

A revolutionary protocol to describe understudied hyperdiverse taxa and overcome the taxonomic impediment

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<http://zoobank.org/FDA33662-0595-4AC1-B9BA-9F2C1311D114>

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Academic editor: D. Zimmermann ♦ Received 21 March 2019 ♦ Accepted 2 July 2019 ♦ Published 25 July 2019

Abstract

Here we elucidate and justify a DNA barcode approach to insect species description that can be applied to name tens of thousands of species of Ichneumonoidea and many other species-rich taxa. Each description consists of a lateral habitus image of the specimen, a COI barcode diagnosis, and the holotype specimen information required by the International Code of Zoological Nomenclature. We believe this approach, or a slight modification of it, will be useful for many other underdescribed hyperdiverse taxa, especially in the tropics. Due to the extreme species-richness of the Ichneumonoidea, the very low percentage of described species, and the lack of detailed biological information for most described species, the standard taxonomic approach is inefficient and overwhelmingly time consuming. A DNA barcode-based approach to initial description will provide a solid foundation of species hypotheses from which more comprehensive descriptions can be developed as other data, time, and budgets permit. Here we elucidate this view and detailed methodology that can generally be applied to species-rich underdescribed taxa. A real example is given by describing species in two genera, *Hemichoma* and *Zelomorpha*, reared from the Área de Conservación Guanacaste in northwestern Costa Rica. The generic type species *Zelomorpha arizonensis* is given a DNA barcode diagnosis and the following new species are described: *Zelomorpha angelsolisi*, *Zelomorpha bobandersoni*, *Zelomorpha danjohnsoni*, *Zelomorpha donwindsori*, *Zelomorpha effugia*, *Zelomorpha johnchemsaki*, *Zelomorpha kellyanneae*, *Zelomorpha larrykirkendalli*, *Zelomorpha mariyavladmirovnae*, *Zelomorpha mikeiviei*, *Zelomorpha myricagaleae*, *Zelomorpha noahjaneae*, *Zelomorpha paulgoldsteini*, *Zelomorpha terryerwini*, *Zelomorpha willsflower-si*, *Hemichoma domwhiteheadi*, *Hemichoma frankhovorei*, and *Hemichoma johnkingsolveri*.

Key Words

Agathidinae, barcode, Braconidae, COI, *Hemichoma*, Hymenoptera, Ichneumonoidea, *Zelomorpha*

Introduction

Systematists have many powerful tools at their disposal for discovering, delimiting and describing new species, and an integrated taxonomic approach, combining morphological characters, identification keys, phylogenetic analyses with multiple molecular markers, and ecological data, is currently the gold standard for quality descrip-

tions of new species (Will et al. 2005; Burns et al. 2008; Pante et al. 2014; Janzen et al. 2017). Such detailed investigation will produce high quality species hypotheses and should be the long-term goal in the taxonomic study of most organisms. However, this approach is highly labor- and resource-intensive, a fact well understood by

those who champion it. When this reality is paired with decreasing manpower and financial support for taxonomic work (Godfray 2007), integrated taxonomic workflows cannot meet the demand for new species documentation produced by the current ecological crises nor by modern technology-assisted exploration of the incredibly species-rich tropics (e.g., Smith et al. 2006, 2008; Fernandez-Triana et al. 2014).

We propose the description of new species based primarily on the DNA barcode molecular marker as a first step in the systematic study of terminal taxa in the highly diverse superfamily Ichneumonoidea. These descriptions are meant to encourage and accelerate 1) the accumulation of additional information on the described species, 2) scientific discussion of the groups treated, 3) opportunities for the refinement of presented species hypotheses as well as those long believed to be established (e.g., Smith et al. 2006), and 4) a species-based framework on which the collateral information of literally hundreds of similar species can be organized. As an example of necessity for a dramatic change in our approach to species descriptions, we elucidate the state of affairs in the Ichneumonoidea below.

The superfamily Ichneumonoidea contains the two most species-rich families of Hymenoptera, i.e., Braconidae and Ichneumonidae. As parasitoids, ichneumonoids exert strong top-down control on their hosts, contributing to ecosystem stability and diversity (Janzen 1981; LaSalle and Gauld 1993; Condon et al. 2014). Many species have economic importance as biological control agents (Sharkey 1997) and display extreme host specificity (Fernandez-Triana et al. 2014).

The Ichneumonoidea contains over 44,000 valid species as of 2016 (Yu et al. 2016). The true number of species can only be crudely estimated, but the superfamily may include as many as 1,000,000. Estimates of total species richness for this group are variable and have increased greatly over recent decades. Dolphin and Quicke (2001) estimated there to be 30,000–50,000 braconid species, using species description rates and comparisons to mammalian diversity patterns. Based on decades of experience working on the morphologically based taxonomy of the family, van Achterberg (in Ghahari et al. 2006) estimated a minimum of 120,000 braconid species in the world, and a roughly equal number of ichneumonids. Rodriguez et al. (2013) used the ratio of described wasp species to Lepidoptera hosts from relatively well-studied sites to estimate the total number of species in the subfamily Microgastrinae, which currently has about 2,000 described species. They predicted between 17,000 and 46,000+ species of Microgastrinae, but noted this was likely an underestimate due to the many undescribed species of Microgastrinae from the well-studied sites used to make the estimations. Extrapolating from the Ichneumonoid database Taxapad (Yu et al. 2016), five of every 100 described ichneumonoid species are microgastrines. Assuming that this ratio holds true for undescribed species and that the estimates made by Rodriguez et al. (2013) are sound, there are between 300,000 and 900,000 species

of Ichneumonoidea. From 2000 to 2011, an average of 468 species of ichneumonoids were described per year (Fig. 1). Given the current rate of species description and these estimates of species diversity, all ichneumonoids, that manage to remain extant, would be described somewhere between the years 2560 and 3842.

Recent revisions of ichneumonoids in the subfamilies Agathidinae and Microgastrinae have investigated the utility of the DNA barcoding region of the gene cytochrome *c* oxidase subunit I (COI) for species delimitation, paired with morphological and ecological host-use characters (e.g., Fernandez-Triana et al. 2014). Kang et al. (2017) created initial molecular operational taxonomic units (MOTUs) for the genus *Lytopylus* using neighbor joining and maximum likelihood trees, clustering species with boundaries at a sequence divergence of 2%. The MOTUs matched the final species concepts for *Lytopylus* at 96.6%. Similarly, revisionary studies of the agathidine genera *Alabagrus* (Sharkey et al. 2018), *Aerophilus* (Sharkey and Chapman 2016), *Euagathis* (Achterberg et al. 2014), *Aphelegathis* (Sharkey et al. 2015), and *Cremnops* (Tucker et al. 2015) used COI data for formation of preliminary MOTUs for species delimitation and found high concordance between MOTUs and final species delimitations. An investigation of the Microgastrinae of Área de Conservación Guanacaste in Costa Rica (again using morphology, COI DNA barcodes, and ecological host data) found all morphological species concepts perfectly corroborated by barcodes (Smith et al. 2008). Additionally, in all of the above cases, DNA barcodes accurately distinguished morphologically cryptic but ecologically distinct species.

While there have been some calls to use molecular species descriptions (Cook et al. 2010; Jörger and Schrödl 2013), few arthropod species have been described based on molecules (Pante et al. 2014) even though DNA sequences are likely more diagnostic than any other trait. There is no stipulation in the International Code of Zoological Nomenclature that prevents or discourages DNA-based descriptions and diagnoses (ICZN 1999). Requirements for the publication of new species include that they be properly named, properly published, have a designated type deposited in an identified place, and that they be accompanied by either a description or diagnosis which can separate them from any known species with which they are likely to be confused. Obviously the diagnosis cannot distinguish them from the many hundreds of species not yet encountered, many of which are now being discovered in modern year-round tropical bioinventory by resident-based biomonitoring (e.g., Janzen and Hallwachs 2016). Barcode-based descriptions are more accurate than morphological descriptions, especially when dealing with diverse, understudied taxa. The COI barcode approach also allows species hypotheses to be posited and data accumulated by employing clear and reproducible methods. By naming these species, we give them a permanent and traceable human-readable record in the literature and electronic databases, and a basis by which they can be back-referenced to information from previous centuries

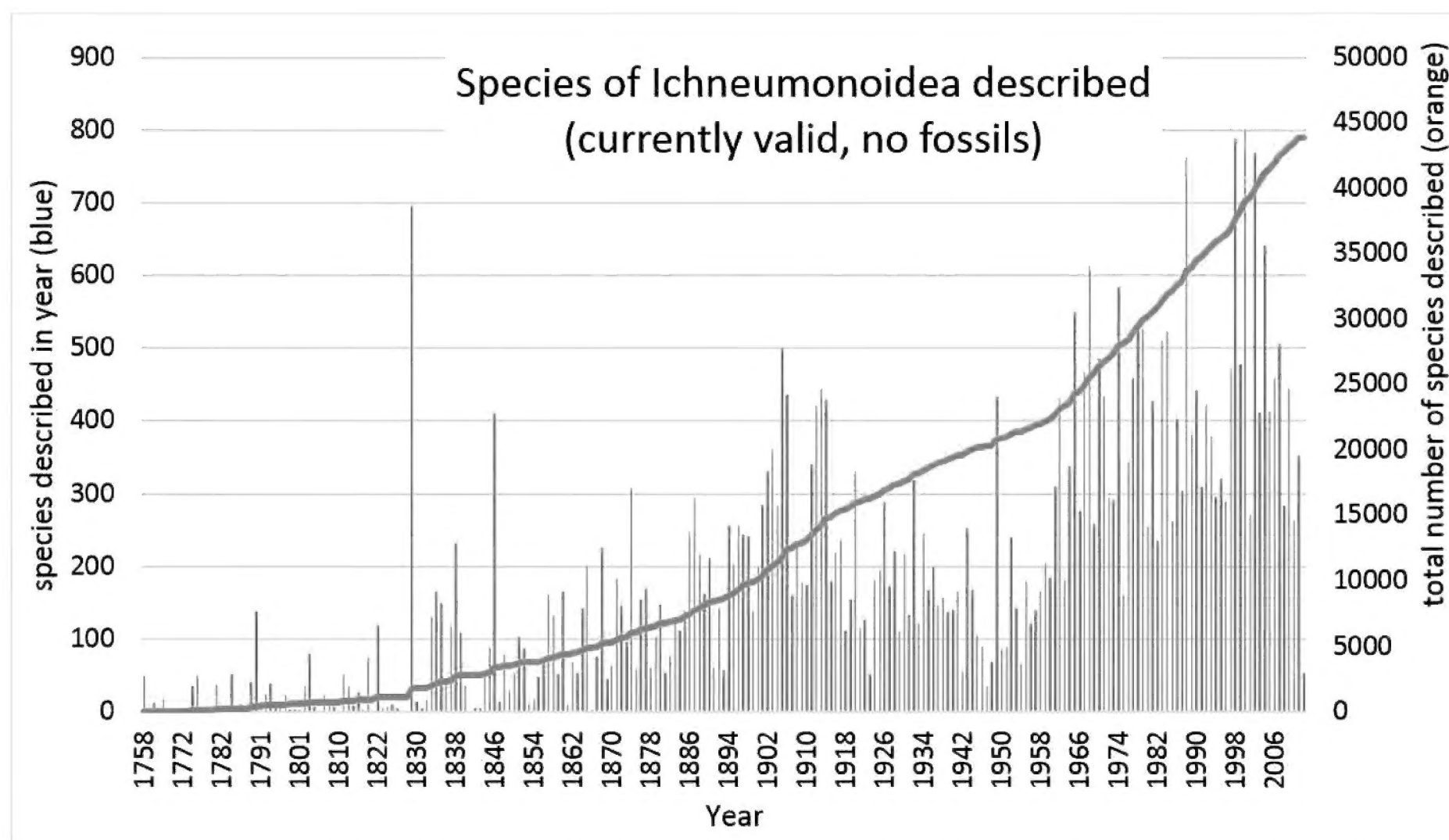


Figure 1. Description rate of Ichneumonoidea species. Data from Taxapad (Yu et al. 2016).

of taxonomic and biological study. Unlike provisional names or simple unique codes, the official names allow the species concepts to be discussed and revised by the scientific community without ambiguity. We do not discourage the standard taxonomic approach. However, we believe that the method outlined here will facilitate such treatments. Furthermore, we find that the barcode-based approach greatly streamlines the ease and accuracy of the standard approach when resources and/or urgency call for it to be applied. We further note that even before standard human-readable names are applied through our suggested barcode-approach, an enormous amount of ecological and natural history study can be conducted with the voucher-based unique codes, and their groupings into BIN codes (Ratnasingham and Hebert 2013) that link the barcode to a scientific name (Janzen and Hallwachs 2016; in prep).

Methods

Specimen collection and documentation

Most specimens were collected by rearing host caterpillars in Área de Conservación Guanacaste (ACG) in north-western Costa Rica (Janzen and Hallwachs 2016). Caterpillar hosts were collected by a team of parataxonomists (Janzen and Hallwachs 2011) as part of the ongoing project to document all ACG non-leaf-mining Lepidoptera larvae, their food plants, and their parasitoids (Janzen and Hallwachs 2016). These caterpillars were databased with collection information, food plant information, and often a photograph, and they were reared to adults. When an

adult moth, butterfly, or parasitoid emerged, the specimen was preserved oven-dried or frozen in 95% ethanol, and a leg was sent to the Centre for Biodiversity Genomics (CBG) (<http://biodiversitygenomics.net>) for DNA barcoding at a cost ranging from \$3–\$10 USD per specimen. Barcodes are deposited with their collateral specimen voucher data in the Barcode of Life Data System (www.boldsystems.org). A generic assignment was confirmed for all specimens of *Hemichoma* and *Zelomorpha* using morphological characters (and both genera form their own monophyletic group in a standard Hymenoptera NJ tree of thousands of species). Focus-stacked images of specimens were taken using a JVC digital camera mounted on a Leica microscope and compiled with the program Automontage. Image post-processing was done in Adobe Photoshop.

DNA extraction and sequencing

Molecular work was carried out at the Centre for Biodiversity Genomics (CBG) using their standard protocols. A leg of each specimen was destructively sampled for DNA extraction using a glass fiber protocol (Ivanova et al. 2006). Extracted DNA was amplified for a 658-bp region near the 5' terminus of the CO1 gene using standard insect primers LepF1 (5'-ATTCAACCAATCATAAAGATATTGG-3') and LepR1 (5'-TAAACTTCTGGATGTC-CAAAAAATCA-3') (Ivanova and Grainger 2007). If initial amplification failed, additional amplifications were conducted following the established protocols using internal primer pairs, LepF1-C113R (130 bp) or LepF1-C-ANTMR1D (307 bp) and MLepF1-LepR1 (407 bp) to

generate shorter overlapping sequences. Amplified products were sequenced using Sanger technology.

Sequence analysis and species determination

Sequences (barcodes) were assigned to operational taxonomic units called barcode index numbers (BINs) that were generated in tandem with the neighbor-joining tree of the barcodes themselves (Appendix 1).

Morphology and host information were compared to BIN assignments that package the NJ tree. Specimen groupings, a BIN, suggested by all data sources were considered to be species. However, for other higher taxa there are cases where several obvious species are packaged into one BIN (e.g., Hebert et al. 2004; Janzen et al. 2017), basically because the BIN algorithm separates groups at about a 2% difference, while in the early stages of evolutionary separation, the species became distinctive before the barcodes attained 2% difference. *Zelomorpha arizonensis*, as discussed below, may be such a case in this study. Type specimens of all previously described *Zelomorpha* and *Hemichoma* species were examined by MJS and his notes were used to verify that *Z. arizonensis* is the sole previously described species in the genus.

Consensus barcodes were created for each species using BioEdit (Hall 1999) and aligned to the *Drosophila melanogaster* complete mitochondrial genome from the NCBI Reference Sequence Database, accession number NC_024511. Consensus barcodes for all species in each genus were compared to all other species in the genus. Nucleotides that were shared by all specimens of a species and by no specimens of any other species were recorded as diagnostic characters. Diagnostic characters are called by their position in the alignment with the *D. melanogaster* reference sequence.

For *Zelomorpha arizonensis*, the only previously described species that we included in the dataset, a number of specimens collected in the southwestern USA and Mexico were included in the NJ tree in addition to the sequences of specimens from ACG.

Specimen information

Holotypes are deposited in the insect collection in the Biology Department of Utah State University in Logan, Utah (EMUS), formerly the insect collection of the American Entomological Institute, Gainesville, Florida. Paratypes are divided between the EMUS and the Hymenoptera Institute Collection (HIC). Detailed specimen records are available on Janzen and Hallwach's database (<http://janzen.sas.upenn.edu/caterpillars/database.lasso>) by searching for specimen voucher codes (DHJPARxxxxxxx), and equally, for a reduced set of specimen collateral in BOLD. Additional specimen information on host caterpillars can be found by search-

ing for their yy-SRNP-xxxxxx voucher codes at <http://janzen.sas.upenn.edu/caterpillars/database.lasso>; there are no data (other than name) for the caterpillar host in BOLD because the cadaver was not barcoded, but rather, identified by its morphology, food plant and other ecological collateral.

Some host species are still awaiting full identification and are given interim names, generally based on their barcodes, just as is the case with the wasps described here. For example, *Hemiceras plusiata*DHJ01 is identified to the genus *Hemiceras* by classical morphology-based criteria. However, it is one of the two reared in a species complex that used to be known as *H. plusiata*. One of the two is the actual *H. plusiata*, and the other is new, but which is which cannot be determined until the holotype is barcoded, or more closely examined morphologically. This was the case with *Udranomia kikkawai*DHJ01, *H. kikkawai*DHJ02 and *H. kikkawai*DHJ03, recently rendered into three species (Janzen et al. 2017). *Udranomia kikkawai* was described from a Venezuelan holotype in 1906. It retained the holotype name through being confirmed by its barcode. When such interim-named species are assigned an official epithet (scientific name) in the future, the barcode and BIN will remain searchable in Janzen's database as well as BOLD, GenBank, and any other public repository. Complete DNA sequence and specimen information is available at [dx.doi.org/10.5883/DS-ASZELO](https://doi.org/10.5883/DS-ASZELO).

Results

Species delimitation

Three hundred thirty-six specimens of *Zelomorpha* and *Hemichoma* with COI barcodes were determined to represent 20 species in two genera by their barcodes, their BINs, and by concomitant morphological inspection. BIN assignments were the same as final species hypotheses (Appendix 1), except in one case in which two species are included in one BIN, i.e., *Z. johnchemsaki* and *Z. bobandersoni*. *Zelomorpha johnchemsaki* parasitizes only *Hemiceras pallidula* (Notodontidae) feeding on *Inga*, while *Z. bobandersoni* parasitizes only on two species of *Hemiceras plusiata* feeding on *Tachigali costaricensis* (Fabaceae) in the same ecosystem. Although these two species have a low interspecific p-distance (2.29%), there is a clear gap between them due to the low variation within species: maximum intraspecific p-distances are 0.30% and 0.16% for *Z. johnchemsaki* and *Z. bobandersoni*, respectively. The separation of these two species is also supported by host plant and host caterpillar differences (Appendix 1). *Hemichoma frankhovorei* contains the greatest intraspecific p-distance and the barcodes are conspicuously variable in length, which obviously results in a high intraspecific p-distance (0.93%), but with no clear subgroupings by morphology, barcode, or ecology (and all parasitize the

same genus of caterpillars). *Zelomorpha arizonensis* is the only previously described species in this dataset. We describe all other species as new.

Systematics

Zelomorpha Ashmead, 1900

Type species. *Zelomorpha arizonensis* (by monotypy) (Ashmead 1900).

Diagnosis. *Zelomorpha* can be distinguished from all other Agathidinae genera by the following combination of morphological characters: fore tarsal claws cleft and not pectinate; foretibial spur shorter than first tarsomere; ovipositor shorter than half the length of the metasoma; frons bordered by carinae; hind trochantellus with one or two longitudinal ridges; notauli variable, usually distinct; gena not elongate.

Biology. The species of *Zelomorpha* are koinobiont solitary endoparasitoids of free-living, late instar medium-small Lepidoptera larvae (Sharkey 1997). Larvae emerge from pre-pupal caterpillars after the caterpillars have spun their cocoons. The parasitoid larvae then spin pale silk cocoons within the host cocoons, next to the host cadavers.

Distribution. *Zelomorpha* occur only in the New World, from the southern USA to Argentina and are pri-

marily Neotropical (Sharkey et al. 2006; Sharkey and Chapman 2017).

Species richness. Including the fifteen new species described here, there are 67 described species of *Zelomorpha* (Yu et al. 2016).

Zelomorpha angelsolisi Meierotto, sp. nov.

<http://zoobank.org/82FC9D54-84E0-470B-8A02-2F80FBFAC5B6>

Figure 2

Molecular diagnosis. Nucleotides 43–45 TTA, 54–57 CTTT, 75 G, 136–138 GTG, 165 T, 321 G, 417 G, 462 G, 477 C, 561 G, 684 G.

Biology. This species has characteristics associated with nocturnal habits: pale coloration, large compound eyes and ocelli. Specimens were reared from caterpillars in the family Erebidae feeding on Fabaceae: *Azeta ceramina* on *Acosmium panamense*, *Chabora repugnalis* DHJ01 on *Indigofera costaricensis*, and *Coenipeta bibitrix* on *Enterolobium cyclocarpum*. Host caterpillars were collected in April, May, and November.

Notes. Many specimens of this species were originally identified as *Zelomorpha arizonensis* by MJS based on morphology. However, p-distances between *Z. arizonensis* and *Z. angelsolisi* are greater than 8%.

Type material. Holotype ♀: DHJPAR0009310 (ASBR577-06), Costa Rica, Área de Conservación Gua-



Figure 2. Lateral image of *Zelomorpha angelsolisi* holotype female.

nacaste, Sector Mundo Nuevo, Punta Plancha, GPS: 10.7416, -85.42734, 420 m elevation, Mariano Pereira coll., reared from *Azeta ceramina* 05-SRNP-56517, host collected 30 May 2005, wasp eclosed 17 June 2005, (EMUS). Paratypes: DHJPAR0009321 (ASBR588-06), DHJPAR0009322 (ASBR589-06), DHJPAR0009314 (ASBR581-06), DHJPAR0009315 (ASBR582-06), DHJPAR0009316 (ASBR583-06), DHJPAR0009313 (ASBR580-06), DHJPAR0009318 (ASBR585-06), DHJPAR0009317 (ASBR584-06), DHJPAR0009311 (ASBR578-06), DHJPAR0009312 (ASBR579-06), DHJPAR0009319 (ASBR586-06), DHJPAR0009320 (ASBR587-06), DHJPAR0009323 (ASBR590-06), DHJPAR0021152 (ASBC964-07), DHJPAR0028276 (ASHYF038-09), DHJPAR0028275 (ASHYF037-09), DHJPAR0015578 (ASAG264-07), DHJPAR0015593 (ASAG279-07), DHJPAR0015584 (ASAG270-07), DHJPAR0015592 (ASAG278-07), DHJPAR0015579 (ASAG265-07), DHJPAR0015577 (ASAG263-07), DHJPAR0015556 (ASAG242-07), DHJPAR0029184 (ASHYE591-09), DHJPAR0015590 (ASAG276-07), DHJPAR0015588 (ASAG274-07).

Etymology. *Zelomorpha angelsolisi* is named in honor of Angel Solis of INBio and the Museo Nacional de Costa Rica, a master taxonomist of Coleoptera and curator who has massively contributed to the inventory of Costa Rican Coleoptera.

Zelomorpha arizonensis Ashmead, 1900.

Figure 3

Molecular diagnosis. Nucleotides 515 C, 648 T

Biology. Adults of this species have characteristics associated with nocturnal habits: pale coloration, large compound eyes and large ocelli. All individuals were reared from *Bulia mexicana* (Erebidae) caterpillars feeding on mature leaves of *Prosopis juliflora* (Fabaceae) at the edge of ACG mangrove swamps in the month of July.

Notes. The host of *Z. arizonensis* from the type locality in the southwestern United States is unknown. However, the range of *Prosopis juliflora* extends northwards through Mexico and into the United States, where it is fed upon by several species of *Bulia*. P-distances between specimens from Costa Rica and the US are close to 1.5% (Fig. 2), which is more than separates many morphologically and ecologically distinctive ACG species (Hebert et al. 2004; Burns et al. 2007; Janzen et al. 2017), including *Z. johnchemsaki* and *Z. bobandersoni*. We have elected to identify this ACG species as being *Z. arizonensis* because of its morphological similarity to *Z. arizonensis* and because its DNA barcode differs from that species by only 1.5% in the sample of six ACG specimens and two Arizona specimens (NJ tree in Appendix 1). However, because there are hundreds of morphologically and ecologically distinctive ACG sympatric or parapatric insect species pairs that show similar or less divergence (e.g., Hebert et

al. 2004; Burns et al. 2007; Janzen and Hallwachs 2016; Janzen et al. 2017), it is possible that two cryptic species will eventually be confirmed within this name, with the Costa Rican species being new.

Material examined. Figured specimen ♀: DHJPAR0052709 (ASHYM2063-13), Costa Rica, Área de Conservación Guanacaste, Sector Santa Rosa, Argelia, GPDS: 10.78004, -85.66405, 5 m elevation, Guillermo Pereira coll., reared from *Bulia mexicana* 13-SRNP-17758, host collected 13 July 2013, wasp eclosed 29 July 2013, (EMUS). Other specimens: Costa Rica: DHJPAR0052704 (ASHYM2058-13), DHJPAR0052702 (ASHYM2056-13), DHJPAR0052703 (ASHYM2057-13), DHJPAR0052708 (ASHYM2062-13; EMUS), DHJPAR0052705 (ASHYM2059-13), DHJPAR0052707 (ASHYM2061-13; HIC). Arizona: HICH015113 (GBMIN73766-17; HIC), HICH015114 (GBMIN142474-18; HIC), BIOUG02486-B12 (BBHYA1354-12), BIOUG02486-C01 (BBHYA1355-12), BIOUG02486-C02 (BBHYA1356-12), BIOUG02580-A06 (BBHYA1778-12), BIOUG02580-B07 (BBHYA1791-12), BIOUG02580-C06 (BBHYA1802-12), BIOUG02580-C08 (BBHYA1804-12), BIOUG02580-C09 (BBHYA1805-12), BIOUG02587-B02 (BBHYA2356-12), BIOUG02587-B03 (BBHYA2357-12), BIOUG02644-H11 (BBHYA3007-12), BIOUG02645-A09 (BBHYA3016-12), BIOUG02645-D12 (BBHYA3055-12), BIOUG02645-E02 (BBHYA3057-12), BIOUG02645-E09 (BBHYA3064-12), BIOUG02645-E10 (BBHYA3065-12), 10BBHYM-0795 (BBHYG795-10), 09BBHYM-158, 09BBHYM-159, 09BBHYM-1106, 09BBHYM-1107, 09BBHYM-1108, 09BBHYM-1109, 09BBHYM-1110, 09BBHYM-1111 (BIOUG). New Mexico: BIOUG02644-G07 (BBHYA2991-12; BIOUG). Texas: 09BBHYM-1112 (BIOUG).

Etymology. *Zelomorpha arizonensis* was named for its holotype locality.

Zelomorpha bobandersoni Meierotto, sp. nov.

<http://zoobank.org/AB75E8BD-A06E-401E-8CAB-060558F66DF1>

Figure 4

Molecular diagnosis. Nucleotides: 72–75 GGGT, 163 G, 222–225 GGGG, 264 G

Biology. All known individuals were reared from *Hemiceras plusiata*DHJ01 or *Hemiceras plusiata*DHJ02 (Notodontidae) feeding on *Tachigali costaricense* (Fabaceae); the caterpillars are indistinguishable without barcoding them. Host caterpillars were collected in January, February, April, and June through October.

Type material. Holotype ♀: DHJPAR0028037 (ASHYE274-08), Costa Rica, Área de Conservación Guanacaste, Sector Pitilla, Estacion Quica, GPS: 10.99697, -85.39666, 470 m elevation, Mauricio Siezar coll., reared from *Hemiceras plusiata*DHJ01 08-SRNP-71265, host collected 10 July 2008, wasp eclosed 11 August 2008, (EMUS). Paratypes: DHJPAR0009346 (ASBR613-06), DHJPAR0009345 (ASBR612-06), DHJPAR0036332



Figure 3. Lateral image of *Zelomorpha arizonensis* female.

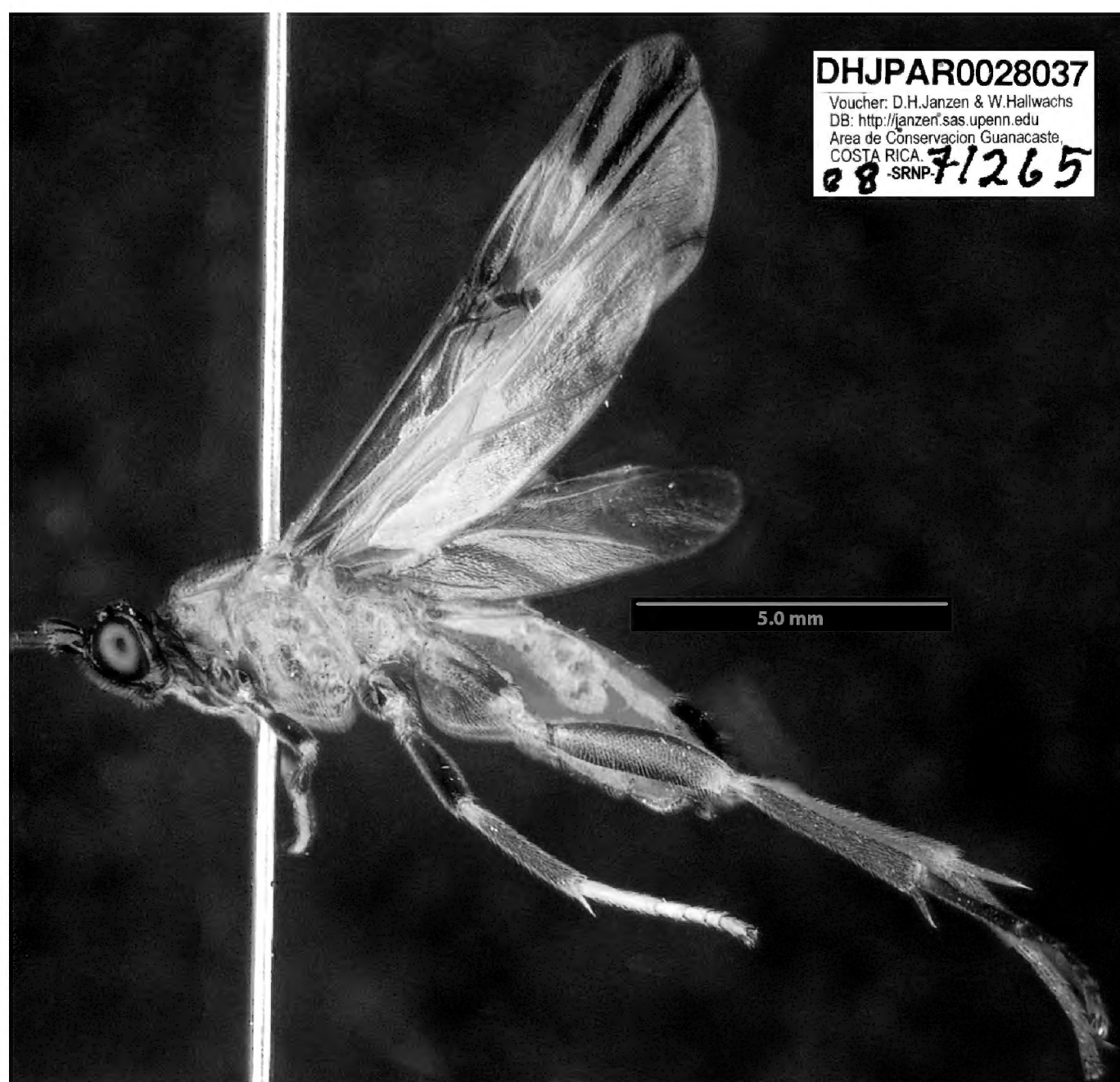


Figure 4. Lateral image of *Zelomorpha bobandersoni* holotype female.

(ASHYD1523-09), DHJPAR0036330 (ASHYD1521-09), DHJPAR0036331 (ASHYD1522-09), DHJPAR0052686 (ASHYM2040-13)

Etymology. *Zelomorpha bobandersoni* is named in honor of Bob Anderson of the Canadian Museum of Nature, Ottawa, in recognition of his taxonomic and curatorial support for understanding the Curculionidae of Costa Rica.

***Zelomorpha danjohnsoni* Meierotto, sp. nov.**

<http://zoobank.org/946520CB-DEB7-437C-84F5-5E2F7C05A3CB>

Figure 5

Molecular diagnosis. Nucleotides: 98 G, 111 G, 264 C, 310 G, 375 A, 452 T, 495 A, 507 G, 513 G, 648 G

Biology. The host of the holotype and one additional specimen lacking COI data were collected in June. Both were reared from *Diastema morata* (Noctuidae) on feeding on *Lantana camara* (Verbenaceae).

Type material. Holotype ♀: DHJPAR0009409 (ASBR676-06), Costa Rica, Área de Conservación Guanacaste, Sector Cacao, Quebrada Otilio, GPS: 10.88996, -85.47966, 550m elevation, Dunia Garcia coll., reared from *Diastema morata* 05-SRNP-45510, host collected 7 June 2005, wasp eclosed 14 July 2005, (EMUS).

Etymology. *Zelomorpha danjohnsoni* is named in honor of C. Dan Johnson (RIP) of Arizona State University, in recognition of his taxonomic support for understanding the Bruchinae (Chrysomelidae) of Costa Rica.

***Zelomorpha donwindsori* Meierotto, sp. nov.**

<http://zoobank.org/77937C61-D022-4671-A5F4-3E67F5ABCCAC>

Figure 6

Molecular diagnosis. Nucleotides: 78 A, 213 C, 243 A, 390 G, 429 G, 456 G, 506–507 CT, 513 T, 585 G, 588 G, 603 C, 636 C, 660 G, 678–679 TG

Biology. The two specimens of this species were reared from caterpillars in the Euteliidae, *Paectes lunodes* feeding on very young leaves of *Ocotea veraguensis* (Lauraceae) and *Paectes fuscescens* feeding on young leaves of the introduced species *Anacardium occidentale* (Anacardiaceae). Host caterpillars were collected in November and July.

Type material. Holotype ♀: DHJPAR0048721 (ACGBA2263-12), Costa Rica, Área de Conservación Guanacaste, Sector El Hacha, Los Almendros, GPS: 11.03226, -85.52776, 290 m elevation, Elieth Cantillano coll., reared from *Paectes fuscescens* 11-SRNP-23258, host collected 15 November 2011, wasp eclosed 9 January 2012, (EMUS). Paratype: DHJPAR0052679 (ASHYM2033-13).

Etymology. *Zelomorpha donwindsori* is named in honor of Don Windsor of the Smithsonian Tropical Research Institute in Panama, a master taxonomist in Chrysomelidae who also contributed to the early development of ACG. The timbers from his original house are part of an ACG caterpillar rearing barn.

***Zelomorpha effugia* Meierotto, sp. nov.**

<http://zoobank.org/7032E109-6070-4617-ADCA-A76C6660E3A6>

Figure 7

Molecular diagnosis. Nucleotides: 46 A, 96–97 TG, 102 T, 124–127 TTAA, 130 G, 285 G, 352–353 TC

Biology. This species has been reared only from *Cosmosoma hercyna* (Erebidae) caterpillars feeding on mature leaves of *Lacistema aggregatum* (Lacistemataceae), and *Lozania pittieri* (Lacistemataceae). Hosts were collected in September, November, January, and February.

Type material. Holotype ♀: DHJPAR0015541 (ASAG227-07), Costa Rica, Área de Conservación Guanacaste, Sector Rincon Rain Forest, Vochysia, GPS: 10.86666, -85.24528, 320 m elevation, Minor Carmona coll., reared from *Cosmosoma hercyna* 05-SRNP-43568, host collected 30 November 2005, wasp eclosed 27 December 2005, (EMUS). Paratypes: DHJPAR0015535 (ASAG221-07), DHJPAR0009432 (ASBR699-06), DHJPAR0009431 (ASBR698-06), DHJPAR0015538 (ASAG224-07), DHJPAR0009381 (ASBR648-06), DHJPAR0009336 (ASBR603-06), DHJPAR0015546 (ASAG232-07), DHJPAR0015552 (ASAG238-07), DHJPAR0009328 (ASBR595-06), DHJPAR0015553 (ASAG239-07), DHJPAR0015547 (ASAG233-07), DHJPAR0009329 (ASBR596-06), DHJPAR0009330 (ASBR597-06), DHJPAR0009331 (ASBR598-06), DHJPAR0009332 (ASBR599-06), DHJPAR0015551 (ASAG237-07), DHJPAR0015550 (ASAG236-07), DHJPAR0009333 (ASBR600-06), DHJPAR0015548 (ASAG234-07), DHJPAR0009334 (ASBR601-06), DHJPAR0015544 (ASAG230-07), DHJPAR0009335 (ASBR602-06), DHJPAR0015545 (ASAG231-07), DHJPAR0015549 (ASAG235-07), DHJPAR0009337 (ASBR604-06), DHJPAR0009338 (ASBR605-06), DHJPAR0009339 (ASBR606-06), DHJPAR0009340 (ASBR607-06), DHJPAR0009341 (ASBR608-06), DHJPAR0009342 (ASBR609-06), DHJPAR0009343 (ASBR610-06), DHJPAR0009379 (ASBR646-06), DHJPAR0009380 (ASBR647-06), DHJPAR0017282 (ASBD387-07), DHJPAR0017281 (ASBD386-07), DHJPAR0017283 (ASBD388-07), DHJPAR0017275 (ASBD380-07), DHJPAR0017278 (ASBD383-07), DHJPAR0017280 (ASBD385-07), DHJPAR0017279 (ASBD384-07), DHJPAR0054489 (ASHYD3654-14), DHJPAR0054516 (ASHYD3681-14), DHJPAR0054472 (ASHYD3637-14), DHJPAR0054473 (ASHYD3638-14), DHJPAR0054481 (ASHYD3646-14), DHJPAR0054479 (ASHYD3644-14), DHJPAR0054484 (ASHYD3649-14), DHJPAR0054483 (ASHYD3648-14), DHJPAR0054477 (ASHYD3642-14), DHJPAR0054475 (ASHYD3640-14), DHJPAR0054482 (ASHYD3647-14), DHJPAR0054476 (ASHYD3641-14), DHJPAR0054478 (ASHYD3643-14), DHJPAR0054474 (ASHYD3639-14), DHJPAR0056359 (MHMYC2439-15), DHJPAR0057453 (ACGBA5363-15), DHJPAR0057454 (ACGBA5364-15), DHJPAR0057455 (ACGBA5365-15), DHJPAR0057456 (ACGBA5366-15), DHJPAR0057452 (ACGBA5362-15), DHJPAR0056979 (ACGBA4889-15).



Figure 5. Lateral image of *Zelomorpha danjohnsoni* holotype female.

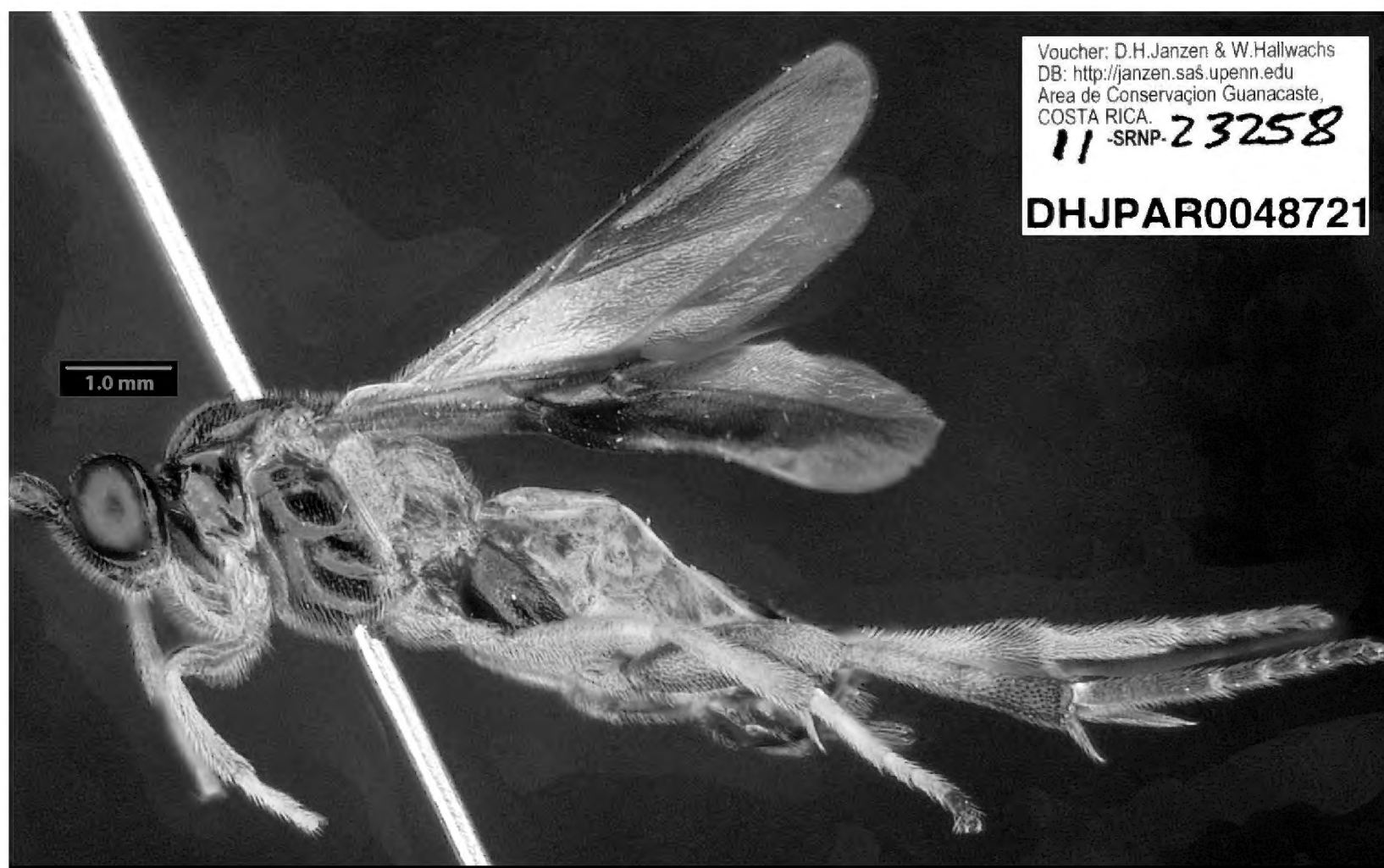


Figure 6. Lateral image of *Zelomorpha donwindsori* holotype female.

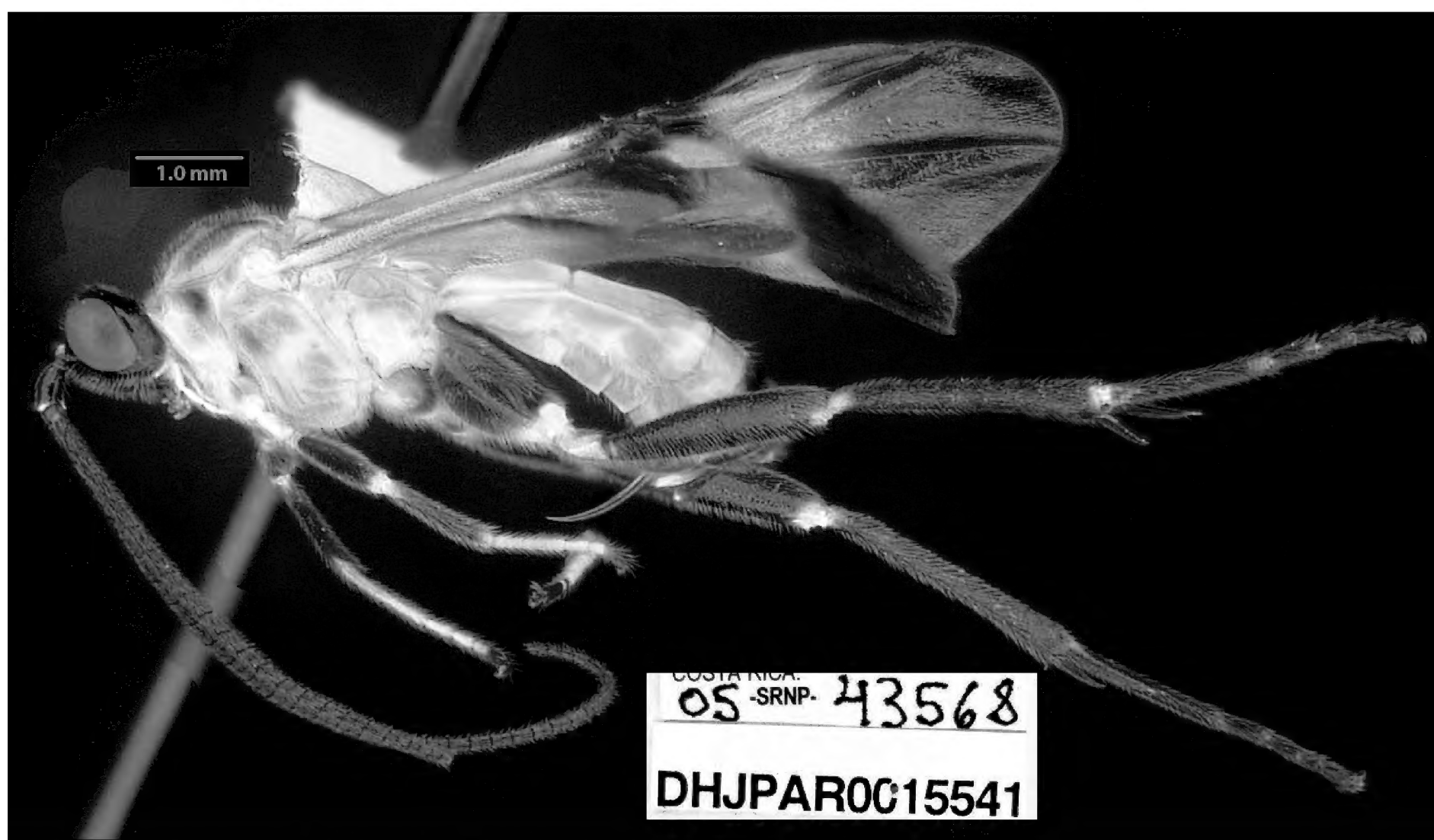


Figure 7. Lateral image of *Zelomorpha effugia* holotype female.

Etymology. *Zelomorpha effugia* is named in honor of the podcast Escape Pod, whose short science fiction stories provided the first author with inspiration and motivation during the production of this manuscript.

***Zelomorpha johnchemsaki* Meierotto, sp. nov.**

<http://zoobank.org/05EA3708-DFE6-4FCD-81B4-33D4EDE323BB>

Figure 8

Molecular diagnosis. Nucleotides: 261 G, 279 C, 537–538 GC, 571 G

Biology. All 24 rearing records for this species are from *Hemiceras pallidula* (Notodontidae) feeding on mature leaves of *Inga vera* and *Inga oerstediana* (Fabaceae). Two of the hosts were collected in October and the others in July.

Notes. Members of *Z. johnchemsaki* are similar to *Z. bobandersoni* in COI sequence and morphology, but the large samples of the two species show consistent differences in color pattern and host preference, and fall in different BINs. They are an excellent example of a shallow split in an NJ tree that definitely represents two species.

Type material. Holotype ♀: DHJPAR0040547 (ASHYE2683-11), Costa Rica, Área de Conservación Guanacaste, Sector Pitilla, Bullas, GPS: 10.9867, -85.38503, 440 m elevation, Ricardo Calero coll., reared from *Hemiceras pallidula* 09-SRNP-71580, host collected 14 July 2009, wasp eclosed 10 August 2009, (EMUS). Paratypes: DHJPAR0023296 (ASHYM048-08), DHJPAR0036326 (ASHYD1517-09), DHJPAR0040539 (ASHYE2675-11), DHJPAR0040536 (ASHYE2672-11),

DHJPAR0040540 (ASHYE2676-11), DHJPAR0040546 (ASHYE2682-11), DHJPAR0040537 (ASHYE2673-11), DHJPAR0040543 (ASHYE2679-11), DHJPAR0040541 (ASHYE2677-11), DHJPAR0036325 (ASHYD1516-09), DHJPAR0040535 (ASHYE2671-11), DHJPAR0036369 (ASHYD1560-09), DHJPAR0040538 (ASHYE2674-11), DHJPAR0040542 (ASHYE2678-11), DHJPAR0040545 (ASHYE2681-11), DHJPAR0040544 (ASHYE2680-11), DHJPAR0036327 (ASHYD1518-09), DHJPAR0036328 (ASHYD1519-09), DHJPAR0036368 (ASHYD1559-09).

Etymology. *Zelomorpha johnchemsaki* is named in honor of John Chemsak (RIP) of the University of California, Berkeley, in recognition of his taxonomic support for understanding the ACG Cerambycidae and teaching DHJ about them in the 1960's.

***Zelomorpha kellyanneae* Meierotto, sp. nov.**

<http://zoobank.org/0C60342A-4476-4526-983A-648B9B1D1C2D>

Figure 9

Molecular diagnosis. Nucleotides: 348 C, 421 A

Biology. This species has been reared only from *Nephodia Janzen18* (Geometridae) feeding on *Heteropterys macrostachya* and *Heteropterys laurifolia* (Malpighiaceae). Host caterpillars were collected in November, February, and May.

Type material. Holotype ♀: DHJPAR0015536 (ASAG222-07), Costa Rica, Área de Conservación Guanacaste, Sector Del Oro, Quebrada Raiz, GPS: 11.02865, -85.48669, 280 m elevation, Lucia Ríos coll., reared from *Nephodia Janzen18*, 05-SRNP-25234, host collected

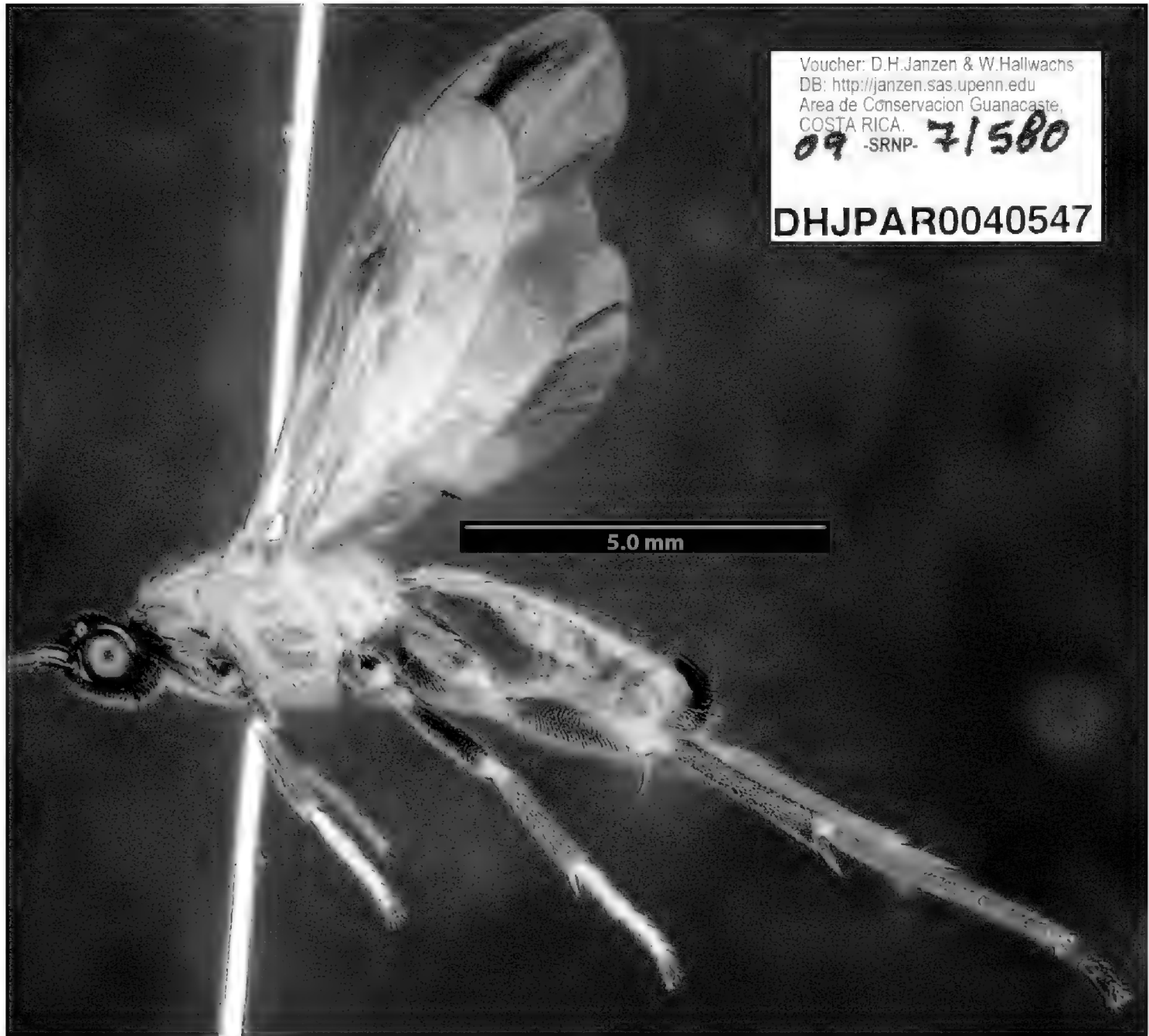


Figure 8. Lateral image of *Zelomorpha johnchemsaki* holotype female.



Figure 9. Lateral image of *Zelomorpha kellyanneae* holotype female.

21 November 2005, wasp eclosed 10 December 2005, (EMUS). Paratypes: DHJPAR0029301 (ASHYE708-09), DHJPAR0009395 (ASBR662-06), DHJPAR0009394 (ASBR661-06), DHJPAR0015543 (ASAG229-07), DHJPAR0015542 (ASAG228-07), DHJPAR0042809 (ASHYH567-11), DHJPAR0042806 (ASHYH564-11).

Etymology. *Zelomorpha kellyanneae* is named in honor of Kelly Meierotto, sister of SM and up and coming archaeologist.

***Zelomorpha larrykirkendalli* Meierotto, sp. nov.**

<http://zoobank.org/14D4279C-C434-45E0-A5C5-2D6148C30406>

Figure 10

Molecular diagnosis. Nucleotides: 81 G, 273 G, 324 T, 369 A, 432 G, 522 A, 662 G

Biology. This species has been reared from three species of *Opisthoxia* (Geometridae) feeding on very young leaves of three species of Primulaceae: *O. molpadia* on *Parathesis glabra*, *O. bella* on *Ardisia compressa*, and *O. uncinata* on *Ardisia auriculata*. Caterpillars were collected in February, March, June, July, and September.

Type material. Holotype ♀: DHJPAR0015540 (ASAG226-07), Costa Rica, Área de Conservación Guanacaste, Sector San Cristobal, Rio Blanco Abajo, GPS: 10.90037, -85.37254, 500 m elevation, Yessenia Men-

doza coll., reared from *Opisthoxia bella* 04-SRNP-4505, host collected 6 September 2004, wasp eclosed 26 September 2004, (EMUS). Paratypes: DHJPAR0055988 (ASHYH2725-14), DHJPAR0055084 (ASHYH1631-14), DHJPAR0052087 (ASHYH1199-13), DHJPAR0055981 (ASHYH2718-14).

Etymology. *Zelomorpha larrykirkendalli* is named in honor of Larry Kirkendall of the University of Bergen, Norway, in recognition of his intense taxonomic interest in Neotropical Scolytidae and Platypodidae, and now, those of ACG.

***Zelomorpha mariyavladmirovnae* Meierotto, sp. nov.**

<http://zoobank.org/029D3E7C-77FA-4F91-8491-70AC1D4E8B98>

Figure 11

Molecular diagnosis. Nucleotides: 250 A, 354 G, 462 C, 543 G

Biology. The single specimen of this species was reared from *Ormetica sicilia* (Erebidae) feeding on mature leaves of *Inga vera* (Fabaceae). Unexpectedly, it appears that this wasp eclosed from the moth pupa rather than from a wasp cocoon spun inside the moth cocoon.

Type material. Holotype ♀: DHJPAR0023528 (ASHYM280-08), Costa Rica, Área de Conservación Guanacaste, Sector Mundo Nuevo, GPS: 10.77175,



Figure 10. Lateral image of *Zelomorpha larrykirkendalli* holotype female.

-85.434, 305 m elevation, Jose Cortez coll., reared from *Ormetica sicilia* 07-SRNP-61364, host collected 28 December 2007, wasp eclosed 14 January 2008, (EMUS).

Etymology. *Zelomorpha mariyavladmirovnae* is named in honor of Mariya Frahm, for her guidance and support to SM.

***Zelomorpha mikeiviei* Meierotto, sp. nov.**

<http://zoobank.org/EC79D207-970B-49F9-AD1F-C9CC1A76B156>

Figure 12

Molecular diagnosis. Nucleotides: 111 C, 411 G, 549 G, 567 G, 661 T

Biology. This species has been reared from three unidentified, different species of host feeding on two different host plants: a species of Geometridae feeding on *Ruellia inundata* (Acanthaceae) and another on *Solanum hayesii* (Solanaceae), and a species of Erebidae feeding on *Colubrina spinosa* (Rhamnaceae). Host caterpillars were collected in January and June.

Type material. Holotype ♀: DHJPAR0029297 (ASHYE704-09), Costa Rica, Área de Conservación Guanacaste, Sector Pitilla, Pasmompa, GPS: 11.01926, -85.40997, 440 m elevation, Calixto Moraga coll., reared from Erebidae 04-SRNP-30170, host collected 12 January 2004, wasp eclosed 6 February 2004, (EMUS). Paratype: DHJPAR0040325 (ASHYE2461-11).

Etymology. *Zelomorpha mikeiviei* is named in honor of Mike Ivie of Montana State University, a master taxonomist in Coleoptera of who has massively contributed to the knowledge base of the inventory of Caribbean Coleoptera and ACG biodiversity inventory.

***Zelomorpha myricagaleae* Meierotto, sp. nov.**

<http://zoobank.org/72D356D0-7744-4C2A-96C5-653CC49588DE>

Figure 13

Molecular diagnosis. Nucleotides: 44 C, 55 A, 64 G, 98 C, 126 C, 135 G, 163 T, 168 G, 183–186 GGTA, 246 C, 258 G, 357–358 GG, 369 G, 381 C, 400–401 AA, 505 T, 519–520 CG, 525 G, 570 A, 603 G, 606 G

Biology. The single specimen of this species was reared from *Speocropia* Poole01 Noctuidae feeding on mature leaves of *Smilax spinosa* (Smilacaceae).

Notes. Known from a single specimen. Holotype is somewhat damaged, missing antennae.

Type material. Holotype ♀: DHJPAR0028033 (ASHYE270-08), Costa Rica, Área de Conservación Guanacaste, Sector Del Oro, Quebrada Trigal, GPS: 11.02681, -85.49547, 290 m elevation, Lucia Ríos coll., reared from *Speocropia* Poole01 08-SRNP-21458, host collected 11 June 2008, wasp eclosed 8 July 2008, (EMUS).

Etymology. *Zelomorpha myricagaleae* is named in honor of Myrica Gale Meierotto, cousin of SM and fierce competitor.

***Zelomorpha noahjaneae* Meierotto, sp. nov.**

<http://zoobank.org/30902D70-7645-4071-A153-0D045619A81D>

Figure 14

Molecular diagnosis. Nucleotides: 108 G, 123 G, 333 G, 519 A, 693 CG

Biology. Specimens of this species were reared from three species of Euteliidae feeding on young leaves of Anacardiaceae: *Paectes fuscescens* on introduced *Anacardium occidentale*, *Eutelia chrysotermia* on *Anacardium excelsum*, and *Paectes* Poole10 on *Mosquitoxylum jamaicense*. Caterpillars were collected in July and November.

Type material. Holotype ♀: DHJPAR0048720 (ACGBA2262-12), Costa Rica, Área de Conservación Guanacaste, Sector El Hacha, Estacion los Almendros, GPS: 11.03226, -85.52776, 290 m elevation, Elieth Cantillano coll., reared from *Paectes fuscescens* 11-SRNP-23262, host collected 15 November 2011, wasp eclosed 30 December 2011, (EMUS). Paratypes: DHJPAR0048723 (ACGBA2265-12), DHJPAR0048719 (ACGBA2261-12), DHJPAR0052678 (ASHYM2032-13), DHJPAR0028023 (ASHYE260-08), DHJPAR0028024 (ASHYE261-08).

Etymology. *Zelomorpha noahjaneae* is named in honor of Noah Jane Meierotto, cousin of SM and an aspiring scientist and possible future entomologist.

***Zelomorpha paulgoldsteini* Meierotto, sp. nov.**

<http://zoobank.org/016912DC-0078-4D19-8472-4DF6F5A9C4A2>

Figure 15

Molecular diagnosis. Nucleotides: 216 G, 327 G, 345–346 AA, 352–354 ACA, 517 C

Biology. This species has been reared from a relatively wide range of hosts in the families Erebidae and Noctuidae, but all hosts feed on leaves of ferns. Caterpillars of paratype specimens were collected in every month except March and April.

Type material. Holotype ♀: DHJPAR0040222 (ASHYE2389-11), Costa Rica, Área de Conservación Guanacaste, Sector Del Oro, Quebrada Serrano, GPS: 11.00025, -85.45614, 585 m elevation, Roster Moraga coll., reared from *Callopistria mexicana* 10-SRNP-21839, host collected 5 August 2010, wasp eclosed 29 August 2010, (EMUS). Paratypes: DHJPAR0044986 (ACGAZ207-11), DHJPAR0057443 (ACGBA5353-15), DHJPAR0057447 (ACGBA5357-15), DHJPAR0057458 (ACGBA5368-15), DHJPAR0057460 (ACGBA5370-15), DHJPAR0015539 (ASAG225-07), DHJPAR0009404 (ASBR671-06), DHJPAR0057649 (ASBR966-15), DHJPAR0030382 (ASHYB1121-09), DHJPAR0054469 (ASHYD3634-14), DHJPAR0054470 (ASHYD3635-14), DHJPAR0054485 (ASHYD3650-14), DHJPAR0036684 (ASHYE1595-09), DHJPAR0028032 (ASHYE269-08), DHJPAR0041152 (ASHYF1067-11), DHJPAR0041153 (ASHYF1068-11), DHJPAR0041159 (ASHYF1074-11), DHJPAR0042357 (ASHYH121-11), DHJPAR0042808



Figure 11. Lateral image of *Zelomorpha mariyavladmirovnæ* holotype female.



Figure 12. Lateral image of *Zelomorpha mikeiviei* holotype female.



Figure 13. Lateral image of *Zelomorpha myricagaleae* holotype female.



Figure 14. Lateral image of *Zelomorpha noahjaneae* holotype female.



Figure 15. Lateral image of *Zelomorpha paulgoldsteini* holotype female.

Table 1. Host caterpillars and their food plants of *Zelomorpha paulgoldsteini*.

Host family	Host species	Food plant family	Food plant species
Erebidae	<i>Nicetas antonalis</i>	Cyatheaceae	<i>Cyathea multiflora</i>
	<i>Nicetas</i> Janzen02	Woodsiaceae	<i>Diplazium myriomerum</i>
	<i>Nicetas</i> Poole22	Dryopteridaceae	<i>Elaphoglossum doanense</i>
	<i>Rejectaria</i> Janzen02	Cyatheaceae	<i>Cyathea multiflora</i>
	<i>Rejectaria</i> Janzen02	Lomariopsidaceae	<i>Lomariopsis vestita</i>
	<i>Rejectaria</i> Janzen06	Cyatheaceae	<i>Alsophila firma</i>
	<i>Rejectaria</i> Poole11	Cyclanthaceae	<i>Cyclanthus bipartitus</i>
	<i>Rejectaria splendida</i>	Cyclanthaceae	<i>Asplundia utilis</i>
	<i>Rejectaria splendida</i>	Cyclanthaceae	<i>Carludovica costaricensis</i>
	<i>Rejectaria splendida</i>	Cyclanthaceae	<i>Asplundia utilis</i>
	<i>Nicetas</i> Poole21	Dryopteridaceae	<i>Didymochlaena truncatula</i>
Noctuidae	<i>Callopistria floridensis</i>	Blechnaceae	<i>Blechnum occidentale</i>
	<i>Callopistria floridensis</i>	Davalliaceae	<i>Nephrolepis biserrata</i>
	<i>Callopistria mexicana</i>	Dryopteridaceae	<i>Bolbitis portoricensis</i>
Erebidae	<i>Nicetas</i> Poole20	Dennstaedtiaceae	<i>Hypolepis repens</i>

(ASHYH566-11), DHJPAR0042810 (ASHYH568-11), DHJPAR0052697 (ASHYM2051-13), DHJPAR0016425 (ASTAP454-06), DHJPAR0016426 (ASTAP455-06).

Etymology. *Zelomorpha paulgoldsteini* is named in honor of Paul Goldstein of the USDA Systematic Entomology Laboratory at the Smithsonian Institution, in honor of his inordinate fondness for the fern-eating caterpillars parasitized by this wasp.

***Zelomorpha terryerwini* Meierotto, sp. nov.**

<http://zoobank.org/01931B54-7D54-4788-8BF5-A706D6104B3C>

Figure 16

Molecular diagnosis. Nucleotides: 66 G, 359 G, 492 C, 621 G

Biology. Hosts of type specimens were collected in January and May through November.



Figure 16. Lateral image of *Zelomorpha terryerwini* holotype female.

Table 2. Host caterpillars and their young leave food plants, for *Zelomorpha terryerwini*.

Host family	Host species	Host plant family	Host plant species
Noctuidae	<i>Cropia cedica</i>	Cordiaceae	<i>Cordia alliodora</i>
	<i>Cropia cedica</i>	Cordiaceae	<i>Cordia panamensis</i>
	<i>Cropia connecta</i>	Cordiaceae	<i>Cordia alliodora</i>
	<i>Cropia europs</i>	Cordiaceae	<i>Cordia alliodora</i>
	<i>Cropia phila</i>	Cordiaceae	<i>Cordia panamensis</i>
	<i>Cropia rivulosa</i>	Cordiaceae	<i>Cordia alliodora</i>
	<i>Cropia rivulosa</i>	Cordiaceae	<i>Cordia panamensis</i>
	<i>Cropia rivulosa</i>	Cordiaceae	<i>Cordia bicolor</i>
	<i>Heterodelta nea</i>	Hypericaceae	<i>Vismia baccifera</i>
	<i>Nephelistis</i> Poole01	Asteraceae	<i>Lepidaploa tortuosa</i>
	<i>Perigea agnonia</i>	Asteraceae	<i>Lepidaploa patens</i>
Nolidae	<i>Iscadia</i> Poole02DHJ03	Hypericaceae	<i>Vismia baccifera</i>

Type material. Holotype ♀: DHJPAR0054486 (ASHYD3651-14), Costa Rica, Área de Conservación Guanacaste, Sector Rincon Rain Forest, Jacobo, GPS: 10.94076, -85.3177, 461 m elevation, Edwin Apu coll., reared from *Iscadia* Poole02DHJ03 13-SRNP-80618, host collected 13 November 2013, (EMUS). Paratypes: DHJPAR0009349 (ASBR616-06), DHJPAR0015554 (ASAG240-07), DHJPAR0022188 (ASTAT1326-07), DHJPAR0023284 (ASHYM036-08), DHJPAR0009420 (ASBR687-06), DHJPAR0009419 (ASBR686-06), DHJPAR0009422 (ASBR689-06), DHJPAR0009421 (ASBR688-06), DHJPAR0015555 (ASAG241-07), DHJPAR0021145 (ASBC957-07), DHJPAR0028156 (ASHYE393-08), DHJPAR0057947 (MHMYK10647-15), DHJPAR0009382 (ASBR649-06), DHJPAR0009383 (ASBR650-06), DHJPAR0009384 (ASBR651-06), DHJPAR0021203 (ASBC1015-07), DHJPAR0053595 (ASHYM2949-13), DHJPAR0054480 (ASHYD3645-14), DHJPAR0009423 (ASBR690-06), DHJPAR0041605

(ASHYF1511-11), DHJPAR0040343 (ASHYE2479-11), DHJPAR0041606 (ASHYF1512-11), DHJPAR0041183 (ASHYF1089-11), DHJPAR0049658 (ASHYB2452-12).

Etymology. *Zelomorpha terryerwini* is named in honor of Terry Erwin of the Smithsonian Institution, a master taxonomist of Coleoptera who has massively contributed to the inventory of Latin American Coleoptera and pesticide-fogged more trees than any other entomologist.

***Zelomorpha willsflowersi* Meierotto, sp. nov.**

<http://zoobank.org/63D1264F-E390-4174-809E-ACE25BBCADBC>
Figure 17

Molecular diagnosis. Nucleotides: 207 G, 303 G, 345 G, 360 G, 398 G, 579 G, 661–663 GTG, 678 G

Biology. This species was reared from three species of Erebidae feeding on young leaves of Fabaceae: *Coenipeta bibitrix* on *Enterolobium cyclocarpum* and *Samanea saman*,

Goniohelio Poole02 on *Senegalia tenuifolia*, and *Tyrissa acygonia* on *Senegalia tenuifolia*. Host caterpillars were collected in May, June, and July.

Type material. Holotype ♀: DHJPAR0009415 (ASBR682-06), Costa Rica, Área de Conservación Guanacaste, Sector Santa Elena, Entrada Santa Elena, GPS: 10.9257, -85.608, 270 m elevation, Elieth Cantillano coll., reared from *Coenipeta bibitrix* 05-SRNP-21918, host collected 5 June 2005, wasp eclosed 22 June 2005, (EMUS). Paratypes: DHJPAR0021205 (ASBC1017-07), DHJPAR0010194 (ASBC475-06), DHJPAR0021146 (ASBC958-07), DHJPAR0009412 (ASBR679-06), DHJPAR0009413 (ASBR680-06), DHJPAR0009414 (ASBR681-06), DHJPAR0009418 (ASBR685-06), DHJPAR0057944 (MHMYK10644-15).

Etymology. *Zelomorpha willsflowersi* is named in honor of Wills Flowers of Florida State University, a master taxonomist of Coleoptera who has massively contributed to the inventory of Costa Rican Chrysomelidae.

Hemichoma Enderlein, 1920

Type species. *Hemichoma fenestratum* Enderlein, 1920.

Diagnosis. *Hemichoma* shares diagnostic morphological characters with *Zelomorpha* except: notauli absent, mesoscutum lacking distinct lobes; gena greatly produced posteroventrally.

Biology. Members of *Hemichoma* are, like *Zelomorpha*, koinobiont endoparasitoids of late instar lepidop-

teran larvae. The solitary wasp larva emerges from the prepupal larva after it has spun its cocoon, and spins its own cocoon inside the host cocoon next to the cadaver.

Distribution. Restricted to the New World, known from Mexico to Argentina.

Species richness. Including the three species described here, there are eight described species of *Hemichoma*.

Hemichoma donwhiteheadi Meierotto, sp. nov.

<http://zoobank.org/E9A8F205-0BB9-426E-A478-74A176B59585>

Figure 18

Molecular diagnosis. Nucleotides: 72 G, 78 G, 90 G, 114 G, 162 T, 168 A, 204 C, 207 G, 216 G, 225 G, 306 G, 318 T, 322 T, 346 G, 357 T, 409–410 GC, 414 G, 492 A, 516 G, 564 A, 585 GC

Biology. All specimens of this species were reared from *Pelochyta misera* (Erebidae: Arctiinae). Food plants include *Heliocarpus appendiculatus* (Malvaceae), the introduced species *Psidium guajava* (Myrtaceae), *Inga oerstediana*, and *Erythrina costaricensis* (Fabaceae). Host caterpillars were collected in June, August, November, and October.

Notes. This species has a sexually dimorphic color pattern: females have bicolored wings and a mostly orange mesosoma, while males have infusate wings and a black mesosoma.

Type material. Holotype ♀: DHJPAR0016918 (ASBR891-07), Costa Rica, Área de Conservación Guanacaste, Sector San Cristobal, Sendero Huerta, GPS: 10.9305,



Figure 17. Lateral image of *Zelomorpha willsflowersi* holotype female.



Figure 18. Lateral image of *Hemichoma donwhiteheadi* holotype female.

-85.37223, 527 m elevation, Elda Araya coll., reared from *Pelochyta misera* 06-SRNP-9643, host collected 27 November 2006, (EMUS). Paratypes: DHJPAR0021147 (ASBC959-07), DHJPAR0016917 (ASBR890-07), DHJPAR0029296 (ASHYE703-09), DHJPAR0022191 (ASTAT1329-07).

Etymology. *Hemichoma donwhiteheadi* is named in honor of Don Whitehead (RIP) of the Smithsonian Institution, a master weevil taxonomist who helped greatly with the taxonomy of ACG Curculionidae.

***Hemichoma frankhovorei* Meierotto, sp. nov.**

<http://zoobank.org/03ECEB9C-16EC-4BBF-93F1-6BAAE30FEDC3>

Figure 19

Molecular diagnosis. Nucleotides: 117 G, 228 C, 243 A, 357 A, 414 A, 477 T, 513 T, 570 A, 615 G, 645 T, 60 A, 663 T

Biology. Multiple species of *Halysidota* (Erebidae; Arctiinae) are the hosts for this wasp: *H. orientalis*, *H. pectenella*, *H. schausi*, and *H. underwoodi* feeding on mature leaves of *Trema micrantha* (Cannabaceae), *Bernardia nicaraguensis* (Euphorbiaceae), and *Acalypha macrostachya* (Euphorbiaceae). Host caterpillars of type specimens were collected between the months of September and December.

Type material. Holotype ♀: DHJPAR0054503 (ASHYD3668-14), Costa Rica, Área de Conservación Guanacaste, Sector Pitilla, Medrano, GPS: 11.01602, -85.38053, 380 m elevation, Ricardo Calero coll., reared from *Halysidota schausi* 13-SRNP-71924, host collected 2 December 2013, wasp eclosed 12 January 2014,

(EMUS). Paratypes: DHJPAR0015563 (ASAG249-07), DHJPAR0030385 (ASHYB1124-09), DHJPAR0030386 (ASHYB1125-09), DHJPAR0037925 (ASHYC4670-10), DHJPAR0037926 (ASHYC4671-10), DHJPAR0054501 (ASHYD3666-14), DHJPAR0054502 (ASHYD3667-14), DHJPAR0036689 (ASHYE1600-09), DHJPAR0036708 (ASHYE1619-09), DHJPAR0036713 (ASHYE1624-09), DHJPAR0028242 (ASHYF004-09), DHJPAR0028243 (ASHYF005-09), DHJPAR0028244 (ASHYF006-09), DHJPAR0028247 (ASHYF009-09), DHJPAR0028248 (ASHYF010-09), DHJPAR0028249 (ASHYF011-09), DHJPAR0028252 (ASHYF014-09), DHJPAR0028254 (ASHYF016-09), DHJPAR0028258 (ASHYF020-09), DHJPAR0028260 (ASHYF022-09), DHJPAR0028263 (ASHYF025-09), DHJPAR0028264 (ASHYF026-09), DHJPAR0041156 (ASHYF1071-11), DHJPAR0041160 (ASHYF1075-11), DHJPAR0041161 (ASHYF1076-11), DHJPAR0029304 (ASHYE711-09).

Etymology. *Hemichoma frankhovorei* is named in honor of Frank Hovore (RIP) of California, a master cerambycid taxonomist who helped greatly with the taxonomic inventory of Costa Rican Cerambycidae.

***Hemichoma johnkingsolveri* Meierotto, sp. nov.**

<http://zoobank.org/9E2998AB-2431-473F-B4B7-835243A6240E>

Figure 20

Molecular diagnosis. Nucleotides: 77 C, 84 G, 108 T, 111 A, 122 C, 141 T, 297 T, 327 G, 357 G, 414 T, 465 A, 579 G, 582 G, 591 G, 648 G, 678 GC



Figure 19. Lateral image of *Hemichoma frankhovorei* holotype female.



Figure 20. Lateral image of *Hemichoma johnkingsolveri* holotype female.

Biology. This species has been reared from *Carathis septentrionalis* (Erebidae) feeding on mature leaves of *Ocotea cernua* (Lauraceae) and *Pachydota saduca* (Erebidae) feeding on several species of *Ocotea* and *Nectandra* (Lauraceae). Host caterpillars of type specimens were collected throughout the year, except between March and May.

Type material. Holotype ♀: DHJPAR0036333 (ASHYD1524-09), Costa Rica, Área de Conservación

Guanacaste, Sector Rincon Rain Forest, Estacion Llanura, GPS: 10.93332, -85.25331, 135 m elevation, Keiner Aragon coll., reared from *Pachydota saduca* 09-SRNP-44900, host collected 4 July 2009, wasp eclosed 8 September 2009, (EMUS). Paratypes: DHJPAR0022195 (ASTAT1333-07), DHJPAR0057457 (ACGBA5367-15), DHJPAR0046730 (ACGBA903-12), DHJPAR0046731 (ACGBA904-12), DHJPAR0046732 (ACGBA905-12),

DHJP0015558 (ASAG244-07), DHJP0015559 (ASAG245-07), DHJP0015560 (ASAG246-07), DHJP0057646 (ASBR963-15), DHJP0038613 (ASHYD2186-10), DHJP0041168 (ASHYF1083-11), DHJP0042358 (ASHYH122-11), DHJP0042359 (ASHYH123-11), DHJP0057945 (MHMYK10645-15), DHJP0058547 (MHMYN8147-16), DHJP0058548 (MHMYN8148-16), DHJP0060427 (ACGBA6848-17), DHJP0060428 (ACGBA6849-17), DHJP0060429 (ACGBA6850-17).

Etymology. *Hemichoma johnkingsolveri* is named in honor of John Kingsolver (RIP) of the USDA Systematic Entomology Laboratory at the Smithsonian Institution, a master taxonomist of Bruchinae (Chrysomelidae) and long-time supporter of ACG biodiversity inventory.

Discussion

Ichneumonoid taxonomists have remained in a taxonomic paradigm that was created for a well-known and largely extra-tropical fauna and flora. For example, there is great utility in a morphological key to the 30 species of butterflies that occur in a suburban backyard in eastern North America; however a key to the 100+ species of 1–4 cm long amber-colored nocturnal highly host-specific species of *Enicospilus* parasitic wasps (Ichneumonidae: Ophioninae) that occur within 3 km of the Administration Area of Área de Conservación Guanacaste is much less useful because, a) they mostly look the same, b) 90% are undescribed, and c) knowing the species name would not give you much additional information, i.e., life history, geographic range, or phenology. Now there is an alternative to morphological keys and complex prose descriptions (Hebert et al. 2003; Ratnasingham and Hebert 2013). The effort and need to create traditional descriptions can be reserved for situations where there is demand for them or until a fairly complete dataset is believed to have been accumulated. However, a species still needs to have a unique identifier, be it a database code or a formal scientific name, so that it can be compared with other species and their collateral.

With online public databases such as BOLD accumulating hundreds of thousands of specimen and species based barcodes (Hebert et al. 2003; <http://ibol.org>), DNA sequences from holotype specimens can be instantly accessed and compared among themselves and with other specimens. If new specimens are delivered with their COI barcodes, taxonomists can rapidly identify new or previously described species. In combination with high quality images and other digitized specimen attributes, online molecular data can enable much revisionary work without the need for physically visiting museums or shipping loaned specimen. Physical collections remain essential as repositories for types, voucher specimens, specimens for further study, and vouchers for the barcodes themselves.

We recognize that DNA barcodes may fail to delimit all species, or all specimens of all species, just as there are

no morphological characters capable of unfailingly separating species. There are drawbacks to using a portion of the single gene COI as a barcode, including potential confusion with nuclear mitochondrial paralogs, *Wolbachia* mediated introgression, hybridization, and incomplete lineage sorting (Rubinoff et al. 2006; Trewick 2008; Calvignac et al. 2011; Klopstein et al. 2016); however, in our opinion, these are trivial problems compared to the efficacy of the approach, and they apply to any other standardized single gene. Most can be overcome by laboratory protocols for recognizing the barcodes alone. Pragmatically, as revealed by the between-species barcode differences for unarguable different species by their morphology and other easy traits, barcodes are easily as reliable for discriminating species as are other traits long used for this purpose and they are superior for discriminating members of tropical complexes of sibling species (e.g., Hebert et al. 2004; Smith et al. 2006, 2008; Burns et al. 2007, 2008, 2010; Bertrand et al. 2014; Janzen et al. 2017).

We demonstrate a novel approach to species descriptions for hyperdiverse, underdescribed taxa, such as those within Ichneumonoidea. The descriptions consist of a high-quality lateral habitus photograph, latitude and longitude coordinates, and the diagnostic characteristics of the COI barcode region, along with details of the holotype as required by the International Code of Zoological Nomenclature (ICZN 1999). The purpose of the lateral habitus images is to allow others who have identified specimens with DNA barcodes to check the plausibility of their determination while they are not meant to assure the species-level identification of specimens based on the images alone. Much more could be included in the species treatments, e.g., extensive images of all body parts, genitalic images (if informative), or SEM images of microsculpture. However, these all take time that is better spent on documenting the tens of thousands of undescribed species. Furthermore, these details will be only poorly informative with respect to the hundreds to thousands of further confamilials that are yet to be discovered in hyperdiverse tropical countries. To effectively solve the problem of the “taxonomic impediment” within Ichneumonoidea, thousands of new species will need to be described annually by many taxonomists. Even with the minimalist approach suggested here, this is a daunting challenge for the future.

Acknowledgments

We gratefully acknowledge the unflagging support of the team of ACG parataxonomists (Janzen et al. 2009; Janzen and Hallwachs 2011) who found and reared the specimens used in this study, and the team of biodiversity managers who protect and manage the ACG forests that are home to these wasps and their caterpillar hosts. The study has been supported by U.S. National Science Foundation grants BSR 9024770 and DEB 9306296, 9400829, 9705072, 0072730, 0515699, and grants from

the Wege Foundation, International Conservation Fund of Canada, Jessie B. Cox Charitable Trust, Blue Moon Fund, Guanacaste Dry Forest Conservation Fund, Area de Conservación Guanacaste, Permian Global, individual donors, and University of Pennsylvania (DHJ and WH). This study has been supported by the Government of Canada through its ongoing support to the Canadian National Collection, and by grants from Genome Canada and Ontario Genomics to PDNH in support of the Centre for Biodiversity Genomics at the University of Guelph, and to the Natural Sciences and Engineering Research Council of Canada.

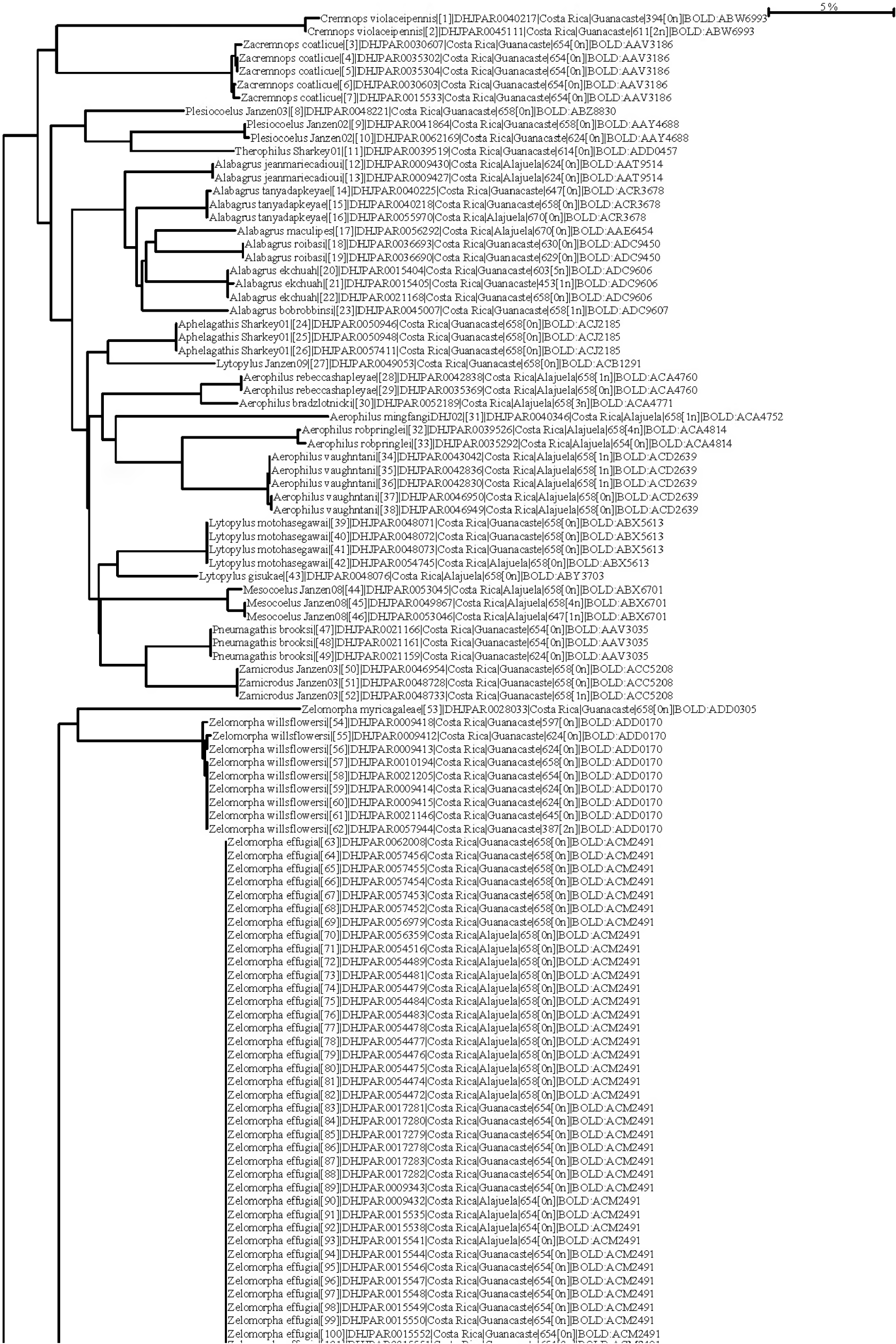
References

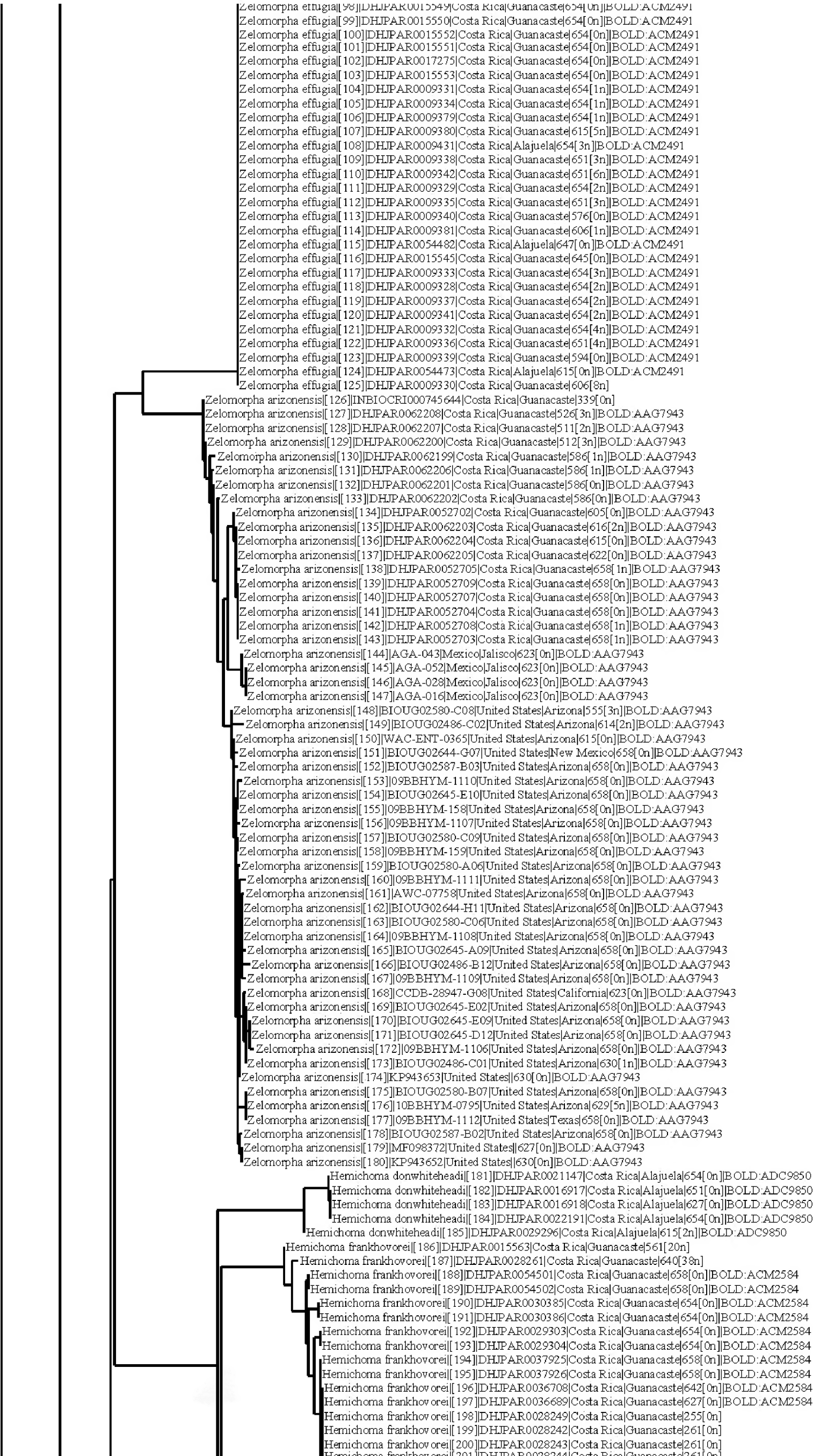
- Achterberg C van, Sharkey MJ, Chapman EG (2014) Revision of the genus *Euagathis* Szépligeti (Hymenoptera, Braconidae, Agathidinae) from Thailand, with description of three new species. *Journal of Hymenoptera Research* 36: 1–25. <https://doi.org/10.3897/jhr.36.5658>
- Ashmead WH (1900) Classification of the ichneumon flies, or the superfamily Ichneumonoidea. *Proceedings of the United States National Museum* 23: 1–220. <https://doi.org/10.5479/si.00963801.23-1206.1>
- Bertrand C, Janzen DH, Hallwachs W, Burns JM, Gibson JF, Shokralla S, Hajibabaei M (2014) Mitochondrial and nuclear phylogenetic analysis with Sanger and next-generation sequencing shows that, in Area de Conservación Guanacaste, northwestern Costa Rica, the skipper butterfly named *Urbanus belli* (family HesperIIDae) comprises three morphologically cryptic species. *BMC Evolutionary Biology* 14: 153. <https://doi.org/10.1186/1471-2148-14-153>
- Burns JM, Janzen DH, Hajibabaei M, Hallwachs W, Hebert PDN (2007) DNA barcodes of closely related (but morphologically and ecologically distinct) species of skipper butterflies (HesperIIDae) can differ by only one to three nucleotides. *Journal of the Lepidopterists Society* 61: 138–153.
- Burns JM, Janzen DH, Hajibabaei M, Hallwachs W, Hebert PDN (2008) DNA barcodes and cryptic species of skipper butterflies in the genus *Perichares* in Area de Conservación Guanacaste, Costa Rica. *Proceedings of the National Academy of Sciences* 105: 6350–6355. <https://doi.org/10.1073/pnas.0712181105>
- Calvignac S, Konecny L, Malard F, Douady CJ (2011) Preventing the pollution of mitochondrial datasets with nuclear mitochondrial paralogs (numts). *Mitochondrion* 11(2): 246–254. <https://doi.org/10.1016/j.mito.2010.10.004>
- Condon MA, Scheffer SJ, Lewis ML, Wharton R, Adams DC, Forbes AA (2014) Lethal Interactions Between Parasites and Prey Increase Niche Diversity in a Tropical Community. *Science* 343(6176): 1240–1244. <https://doi.org/10.1126/science.1245007>
- Cook LG, Edwards RD, Crisp MD, Hardy NB (2010) Need morphology always be required for new species descriptions? *Invertebrate Systematics* 24(3): 322–326. <https://doi.org/10.1071/IS10011>
- Dolphin K, Quicke DL (2001) Estimating the global species richness of an incompletely described taxon: an example using parasitoid wasps (Hymenoptera: Braconidae). *Biological Journal of the Linnean Society* 73(3): 279–286. <https://doi.org/10.1111/j.1095-8312.2001.tb01363.x>
- Fernandez-Triana JL, Whitfield JB, Rodriguez JJ, Smith MA, Janzen DH, Hallwachs W, Hajibabaei M, Burns JM, Solis MA, Brown J, Cardinal S, Goulet H, Hebert PDN (2014) Review of *Apanteles* sensu strictu (Hymenoptera, Braconidae, Microgastrinae) from Area de Conservación Guanacaste, northwestern Costa Rica, with keys to all described species from Mesoamerica. *ZooKeys* 383: 1–565. <https://doi.org/10.3897/zookeys.383.6418>
- Ghahari H, Yu D, Achterberg C van (2006) Bibliography of the family Braconidae (Hymenoptera: Ichneumonoidea) (1964–2003). *NNM Technical Bulletin* 8: 1–293.
- Godfray HCJ (2007) Linnaeus in the information age. *Nature* 446(7133): 259–260. <https://doi.org/10.1038/446259a>
- Hall TA (1999) BioEdit: a user-friendly biological sequence alignment editor and analysis program for Windows 95/98/NT. *Nucleic Acids Symposium Series* 41: 95–98.
- Hanson P, Janzen D, Knapp S, Scoble M, Wahl D, West-Eberhard MJ (2009) Obituary. Ian Gauld FLS (1947–2009). Master taxonomist and morphologist. *The Linnean, Newsletter and Proceedings of the Linnean Society of London* 25(2): 46–47.
- Hebert PDN, Cywinska A, Ball SL, deWaard JR (2003) Biological identifications through DNA barcodes. *Proceedings of the Royal Society of London B, Biological Sciences* 270: 313–321. <https://doi.org/10.1098/rspb.2002.2218>
- Hebert PDN, Penton EH, Burns JM, Janzen DH, Hallwachs W (2004) Ten species in one: DNA barcoding reveals cryptic species in the Neotropical skipper butterfly *Astraptes fulgerator*. *Proceedings of the National Academy of Sciences of the United States of America* 101(41): 14812–14817. <https://doi.org/10.1073/pnas.0406166101>
- ICZN (1999) International Code of Zoological Nomenclature. Fourth Edition. The International Trust for Zoological Nomenclature, London, 306 pp.
- Ivanova NV, Dewaard JR, Hebert PD (2006) An inexpensive, automation-friendly protocol for recovering high-quality DNA. *Molecular Ecology Resources* 6(4): 998–1002. <https://doi.org/10.1111/j.1471-8286.2006.01428.x>
- Ivanova NV, Grainger CM (2007) CCDB protocols, COI amplification. [Online] http://ccdb.ca/docs/CCDB_Amplification.pdf
- Ivanova NV, Grainger CM (2007a) CCDB protocols, COI amplification. http://www.dnabarcoding.ca/CCDB_DOCS/CCDB_Amplification.pdf [accessed 15 July 2010]
- Janzen DH (1981) The peak in North American ichneumonid species richness lies between 38 and 42 degrees North latitude. *Ecology* 62: 532–537. <https://doi.org/10.2307/1937717>
- Janzen DH, Burns JM, Cong Q, Hallwachs W, Dapkey T, Manjunath R, Hajibabaei M, Hebert PDN, Grishin NV (2017) Nuclear genomes distinguish cryptic species suggested by their DNA barcodes and ecology. *Proceedings of the National Academy of Sciences of the United States of America* 114(31): 8313–8318. <https://doi.org/10.1073/pnas.1621504114>
- Janzen DH, Hallwachs W, Blandin P, Burns JM, Cadiou J, Chacon IS, Dapkey T, Deans AR, Epstein ME, Espinoza B, Franclemont JG, Haber WA, Ibabaei MH, Hall JP, Hebert PDN, Gauld ID, Harvey DJ, Hausmann A, Kitching IJ, Lafontaine D, Landry J-F, Lemaire C, Miller JE, Miller JS, Miller L, Miller SE, Montero J, Munroe E, Green SR, Ratnasingham S, Rawlins JE, Robbins RK, Rodriguez JJ, Rougerie R, Sharkey MJ, Smith MA, Solis MA, Sullivan JB, Thiaucourt P, Wahl DB, Weller SJ, Whitfield JB, Willmott KR, Wood DM, Woodley NE, Wilson JJ (2009) Integration of DNA barcoding into an ongoing inventory of complex tropical biodiversity. *Molecular Ecology Resources* 9 (Supplement 1): 1–26. <https://doi.org/10.1111/j.1755-0998.2009.02628.x>

- Janzen DH, Hallwachs W (2011) Joining inventory by parataxonomists with DNA barcoding of a large complex tropical conserved wildland in northwestern Costa Rica. PLoS ONE 6(8): e18123. <https://doi.org/10.1371/journal.pone.0018123>
- Janzen DH, Hallwachs W (2016) DNA barcoding the Lepidoptera inventory of a large complex tropical conserved wildland, Area de Conservación Guanacaste, northwestern Costa Rica. Genome 59: 641–660. <https://doi.org/10.1139/gen-2016-0005>
- Janzen DH, Burns JM, Cong Q, Hallwachs W, Dapkey T, Manjunath R, Hajibabaei M, Hebert PDN, Grishin NV (2017). Nuclear genomes distinguish cryptic species suggested by their DNA barcodes and ecology. Proceedings of the National Academy of Sciences 114(31): 8313–8318. <https://doi.org/10.1073/pnas.1621504114>
- Jörger KM, Schrödl M (2013) How to describe a cryptic species? Practical challenges of molecular taxonomy. Frontiers in Zoology 10(1): 59. <https://doi.org/10.1186/1742-9994-10-59>
- Kang I, Chapman EG, Janzen DJ, Hallwachs W, Solis MA, Sharkey MJ (2017) Revision of the species of *Lytopylus* from Area de Conservación Guanacaste, northwestern Costa Rica (Hymenoptera, Braconidae, Agathidinae). ZooKeys 721: 93–158. <https://doi.org/10.3897/zookeys.721.20287>
- Klopfstein S, Kropf C, Baur H (2016) *Wolbachia* endosymbionts distort DNA barcoding in the parasitoid wasp genus *Diplazon* (Hymenoptera: Ichneumonidae). Zoological Journal of the Linnean Society 177: 541–557. <https://doi.org/10.1111/zoj.12380>
- LaSalle J, Gauld ID (Eds) (1993) Hymenoptera and Biodiversity. CAB International, Wallingford, 368 pp.
- Pante E, Schoelinc C, Puillandre N (2014) From integrative taxonomy to species description: one step beyond. Systematic Biology 64(1): 152–160. <https://doi.org/10.1093/sysbio/syu083>
- Quicke DL (2012) We know too little about parasitoid wasp distributions to draw any conclusions about latitudinal trends in species richness, body size and biology. PLoS ONE 7(2): e32101. <https://doi.org/10.1371/journal.pone.0032101>
- Ratnasingham S, Hebert PD (2013) A DNA-based registry for all animal species: The Barcode Index Number (BIN) System. PLoS ONE 8(7): e66213. <https://doi.org/10.1371/journal.pone.0066213>
- Rodriguez JJ, Fernández-Triana JL, Smith MA, Janzen DH, Hallwachs W, Erwin TL, Whitfield JB (2013) Extrapolations from field studies and known faunas converge on dramatically increased estimates of global microgastrine parasitoid wasp species richness (Hymenoptera: Braconidae). Insect Conservation and Diversity 6(4): 530–536. <https://doi.org/10.1111/icad.12003>
- Rubioff D, Cameron S, Will K (2006) A genomic perspective on the shortcomings of mitochondrial DNA for “barcoding” identification. Journal of Heredity 97(6): 581–594. <https://doi.org/10.1093/jhered/esl036>
- Sharkey MJ (1997) Subfamily Agathidinae. In: Wharton R, Sharkey MJ, Marsh PM (Eds) Manual of the New World genera of the family Braconidae (Hymenoptera). Special publication of the International Society of Hymenopterists 1. The International Society of Hymenopterists, Washington, DC, 68–83.
- Sharkey MJ, Chapman EG (2016) Revision of *Aerophilus* Szépligeti (Hymenoptera, Braconidae, Agathidinae) from eastern North America, with a key to the Nearctic species. Contributions in Science 524: 51–110.
- Sharkey MJ, Chapman EG (2017) Phylogeny of the Agathidinae (Hymenoptera: Braconidae) with a revised tribal classification and the description of a new genus. Proceedings of the Entomological Society of Washington 119: 823–842. <https://doi.org/10.4289/0013-8797.119.SpecialIssue.823>
- Sharkey MJ, Chapman EG (2018) Revision of *Zosteragathis* Sharkey of Thailand (Hymenoptera, Braconidae, Agathidinae, Agathidini). Deutsche Entomologische Zeitschrift 65(2): 225–253. <https://doi.org/10.3897/dez.65.25772>
- Sharkey MJ, Chapman EG, Janzen DH, Hallwachs W, Smith MA (2015) Revision of *Aphelagathis* (Hymenoptera, Braconidae, Agathidinae, Agathidini). Zootaxa 4000(1): 73–89. <https://doi.org/10.11646/zootaxa.4000.1.3>
- Sharkey MJ, Laurence NM, Sharanowski B, Quicke DLJ, Murray D (2006) Revision of the Agathidinae (Hymenoptera: Braconidae) with comparisons of static and dynamic alignments. Cladistics 22: 546–567. <https://doi.org/10.1111/j.1096-0031.2006.00121.x>
- Sharkey MJ, Meierotto S, Chapman EG, Janzen DJ, Hallwachs W, Dapkey T, Solis MA (2018) *Alabagrus* Enderlein (Hymenoptera, Braconidae, Agathidinae) species of Costa Rica, with an emphasis on specimens reared from caterpillars in Area de Conservación Guanacaste. Contributions in Science 526: 31–180. <https://doi.org/10.3897/zookeys.130.1569>
- Smith MA, Woodley NE, Janzen DH, Hallwachs W, Hebert PDN. (2006) DNA barcodes reveal cryptic host-specificity within the presumed polyphagous members of a genus of parasitoid flies (Diptera: Tachinidae). Proceedings of the National Academy of Sciences 103: 3657–3662. <https://doi.org/10.1073/pnas.0511318103>
- Smith MA, Rodriguez JJ, Whitfield JB, Deans AR, Janzen DH, Hallwachs W, Hebert PDN (2008) Extreme diversity of tropical parasitoid wasps exposed by iterative integration of natural history, DNA barcoding, morphology, and collections. Proceedings of the National Academy of Sciences of the United States of America 105(34): 12359–12364. <https://doi.org/10.1073/pnas.0805319105>
- Trewick SA (2008) DNA Barcoding is not enough: mismatch of taxonomy and genealogy in New Zealand grasshoppers (Orthoptera: Acrididae). Cladistics 24(2): 240–254. <https://doi.org/10.1111/j.1096-0031.2007.00174.x>
- Tucker EM, Chapman EG, Sharkey MJ (2015) A revision of the New World species of *Cremnops* Förster (Hymenoptera: Braconidae: Agathidinae). Zootaxa 3916(1): 1–83. <https://doi.org/10.11646/zootaxa.3916.1.1>
- Will KW, Mishler BD, Wheeler QD (2005) The perils of DNA barcoding and the need for integrative taxonomy. Systematic Biology 54(5): 844–851. <https://doi.org/10.1080/10635150500354878>
- Yu DSK, van Achterberg C, Horstmann K (2016) Taxapad, Ichneumonoidea. Vancouver. <http://www.taxapad.com>

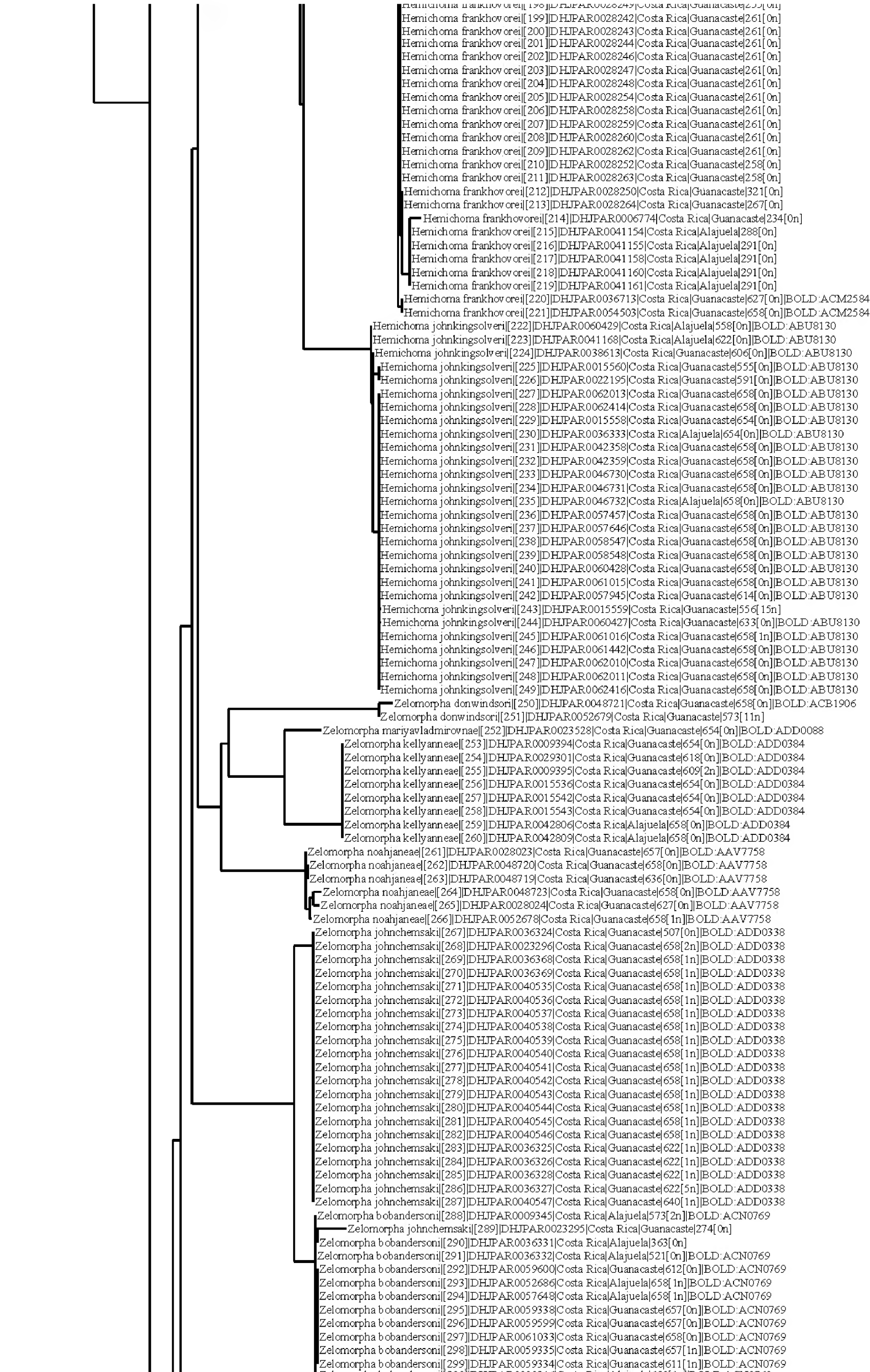
Appendix 1

Neighbor joining (NJ) tree of *Zelomorpha* and *Hemichoma*. The distance model employed was the Kimura 2 parameter.

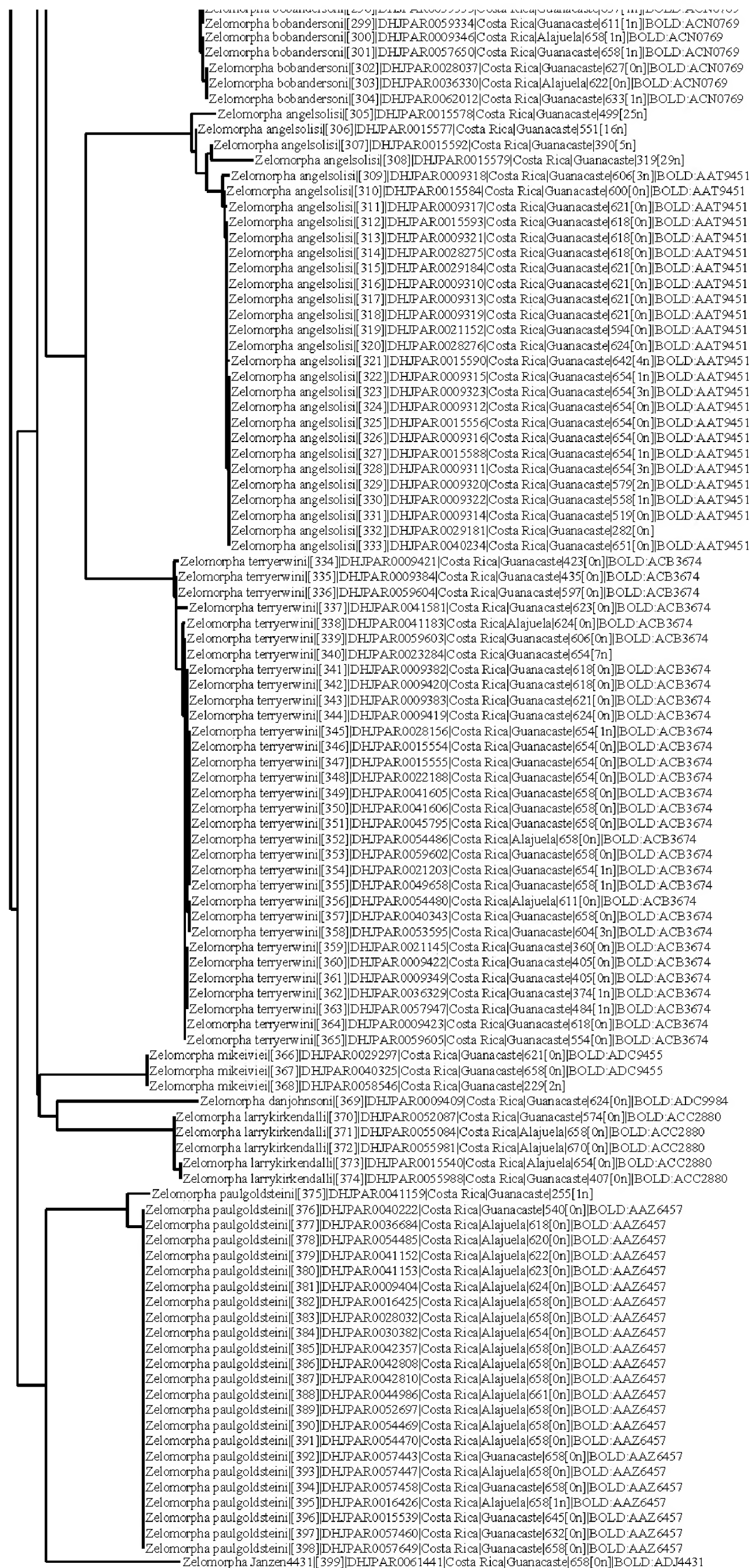




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